



# Don’t Worry, Be Happy: Enhancing the Positive Mood of Older Adults and Investigating Influenza Vaccination Responses

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*Abstract*

Vaccinations are vital for reducing the risk of disease, where they play an important role in combating influenza. However, some populations, such as older adults (≥65 years), respond poorly to influenza vaccination due to their compromised immune systems, with efficacy at 17-53% compared to 70-90% in young adults. This is a phenomenon referred to as immunosenescence, whereby older adults have sub-optimal immune systems and are therefore more susceptible to infectious disease. The burden of influenza is becoming an increasingly important challenge to tackle with the rapidly ageing population in the UK. Every year the UK experiences 4,000 to 14,000 deaths due to influenza, where a staggering 90% of these are older adults.

Pharmaceutical avenues have been explored to improve vaccination, such as the addition of vaccine adjuvants, in the hope that individuals will present greater immune responses and, in turn, improve vaccine effectiveness. Pharmaceutical methods have been proven to improve vaccine efficacy. However, they have not improved the immune responses of older adults to the same degree as young adults, therefore highlighting the need for alternative methods to improve vaccine effectiveness for this vulnerable population. Psychological effects, such as mood, loneliness and depression have been identified as immune modulators, where they have been observed to alter immune responses in a variety of ways. Previous research has also demonstrated that psychological interventions are capable of altering these psychological factors to influence immune responses, such as antibody responses to vaccination. This has highlighted the potential for psychological interventions to be used within a clinical setting to improve the vaccination outcomes of older adults. A previous pilot study (*n*= 138) demonstrated the use of a brief positive mood intervention (standardised, fixed-content intervention) to be effective at enhancing the positive mood of older adults immediately prior to influenza vaccination. In addition, previous research has shown interventions that permit a degree of choice for the participant can result in increased engagement and interest and therefore suggests they could be more effective than non-choice interventions for altering psychological factors, such as positive mood.

This thesis presents a large, a three armed, parallel, randomisation control trial (*n*= 654), which aimed to build upon the body of research investigating positive mood and influenza vaccination responses in older adults. The primary aim of the Flu and Mood in Older Adults (For-ME) trial presented in this thesis was to determine whether a standardised (fixed-content) intervention and/or choice intervention were capable of improving the positive of older adults immediately prior to influenza vaccination, compared with usual care. A

comparison between both intervention types was also carried out to determine whether the standardised or choice intervention was superior for improving positive mood. The secondary aim was to investigate antibody responses to vaccination to determine whether group allocation was a significant predictor of antibody responses to vaccination. Antibody responses to vaccination were measured via Enzyme-Linked Immunosorbent (ELISA) assay and Hemagglutinin Inhibition (HAI) assay and the findings from these two methods were explored and compared.

The research outlined in this thesis revealed that both brief psychological interventions were capable of improving the mood of older adults in a clinical setting, compared with usual care. Both positive mood interventions were equally effective at improving the mood of older adults prior to influenza vaccination. Thus, the provision of choice did not notably enhance the intervention's impact on mood. Antibody responses 4-weeks post-vaccination were found to be robust with seroprotection rates of >80% for all three influenza vaccine strains and across all three arms of the trial. However, antibody responses to vaccination were found to not differ significantly differ between groups. It is concluded that, in the context of highly immunogenic adjuvanted vaccines, any impact of short interventions on subsequent peak antibody responses to influenza vaccination were potentially too small to be detected in the current trial.

Future directions for research can aim to build upon these findings to investigate whether positive mood is capable of acting on the immune system beyond antibody responses to vaccination by considering T cell responses. More importantly, an immune mechanism study is required to drive this area of research forward to gain insight into the types of immune responses altered in the context of positive mood.

*Acknowledgments*

The last three and a half years have without a doubt been the most challenging years of my life to date, from both an academic and personal perspective. On many occasions I have doubted my ability to make it to the end of this journey and finally see the end of this very long PhD tunnel. No one could have ever predicted the challenge of doing a PhD would be made even more difficult by the impact of a pandemic turning the lives of everyone around the world upside-down. Without my incredible support network of family and friends, I would never have been able to make it through the pandemic and to this end of this PhD journey.

To my family scattered around the world, thank you all for your support in so many ways. Dad, thank you for your ongoing support and guidance throughout my whole academic career. You have always been there to offer a listening ear and be the voice of reason when times were tough. I will never have the words to sufficiently express my gratitude. To my Aunt Lisa and Granny in New Zealand sending me care packages, thank you. I hope we will be reunited again soon.

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## *COVID-19 Impact Statement*

This statement aims to outline the disruption caused by COVID-19 and how it impacted on the research presented in this thesis.

As a consequence of the COVID-19 restrictions in March 2020, my laboratory was closed immediately. Around the time laboratory closures were implemented, I was in the process of optimising an ELISA assay for the detection for antibody responses to vaccination. After several months, my laboratory reopened and these optimisation experiments could be resumed (October/ November 2020). However, equipment and safety measures resulted in changes in experimental protocols and further delays in research (more details of these experimental delays and changes are outlined in *Chapter 4*). Overall, these COVID-19 restrictions had a huge impact in terms laboratory research delays.

In light of these unforeseen circumstances, the amount of data presented within this thesis is less than originally planned. Planned research as part of my PhD, that was disrupted by COVID-19, included an investigating into the immunological mechanisms behind the immune enhancement of positive mood. This research study was unable to go ahead due to non-COVID-19 clinical research being halted indefinitely. Unfortunately, the data that would have been collected as part of this study would have contributed a critical piece of novel research to this thesis. Sadly, this important study was not conducted as part of my PhD but has instead been suggested as a future research study to expand upon the research presented in this thesis. More details of this study are outlined towards the backend of this thesis, in the "*Future Research*" section of *Chapter 6*.

Additional difficulties experienced as a result of COVID-19 include mental health challenges and being unable to see family for a long period of time. The toll of the stress of the pandemic and being unable to see family, due to them living abroad, resulted in me reaching out for mental health support. For a total of 4-months, I received mental health support in the form of private therapy (April to July 2020). This period of time was extremely challenging, where I was receiving weekly therapy sessions and faced difficulties with working from home due to moving into a new house with the family of a friend to avoid living on my own. In an attempt to mitigate the effect of the pandemic on laboratory work, this time was dedicated to thesis writing, which was hindered considerably due to these difficulties. The implication of this meant that whilst some thesis writing was done, the quantity and quality of the produced work was less that what would have otherwise been delivered under normal circumstances.

Despite these PhD disruptions, I was given the opportunity to contribute towards COVID-19 research via my involvement in 2 separate studies. My contribution to these projects lasted 8-months in total, from January to August 2021. The first study I was involved in investigated T-cell mediated immune responses in asymptomatic COVID-19-positive patients. My role in this project was to assist with processing blood and saliva samples in the laboratory, where I primarily focused on the isolation of peripheral blood mononuclear cells (PBMCs). I also played a key role in the clinic, where I helped consent study participants and took patient blood samples (I was phlebotomy trained for this role). The second study investigated behavioural and psychological influences on the immune system, where I was primarily based in the clinic to consent participants and assist with the fluid flow of study activities. This was an incredibly rewarding opportunity for me to get involved in important and exciting research. It was also a valuable use of my time whilst being unable to carry out the above-mentioned planned PhD research.

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# 1 Chapter 1: Global Impact of Infectious Disease and the Importance of Vaccination

## 1.1 Chapter Introduction

This chapter aims to introduce the reader to vaccination, which is the main focus of this thesis. Vaccinations are considered one of the greatest breakthroughs of modern medicine and are critical for controlling and, in some cases, eradicating infectious diseases. This chapter outlines the global impact of infectious disease, with a particular focus on influenza. Influenza has had a substantial social and economic burden in the western world, whereby older adults are disproportionally affected due to their naturally declining immune systems. Pharmaceutical avenues for improving the effectiveness of the influenza vaccine have been explored, such as vaccination adjuvants, but have had limited success for the older adult population (McKee et al., 2007). As a result, there is a growing interest in behavioural and psychological influences on immune modulation, whereby psychological interventions have shown promise as an avenue for improving vaccine effectiveness and is described in more detail in *Chapter 2* of this thesis.

## 1.2 The Burden of Infectious Disease

Infectious diseases are a major worldwide problem, where they have had an immense impact on public health and the economy (K. Smith et al., 2019). The occurrence of infectious diseases is due to the transmission of pathogenic microorganisms, such as bacteria, viruses, parasites or fungi, that spread from person to person (van Doorn, 2014). Infectious diseases are important causes of morbidity and mortality worldwide, with current rates at 3 million and 5 hundred thousand, respectively (Paget et al., 2019). They account for nearly a quarter of worldwide mortalities, disproportionally impacting vulnerable populations. These populations include older adults with sub-optimally functioning immune systems and poverty-rife populations who lack the resource to deal with disease's frequent occurrence (Bhutta et al., 2014; Lozano et al., 2012). For example, research has indicated that older adults have an influenza vaccination response range of 18-50% (Govaert, 1994; P. A. Gross et al., 1995) compared to 70% observed in younger adults (Bernstein et al., 1999). Conventionally, individuals classified as responders to influenza vaccination (also referred to as seroprotected in the literature and throughout this thesis) display antibody levels above a predefined threshold (e.g., titre of >40 measured by hemagglutinin inhibition assay). The most prevalent infectious diseases based on total annual deaths include Tuberculosis,

HIV/AIDS, Malaria, Influenza, *E.coli*, Typhoid Fever, Measles, Shigellosis, Whooping Cough, Tetanus, Cholera and Rabies (Lozano et al., 2012).

Unfortunately, this global issue does not seem to be improving. In the last two decades, 16 new infectious diseases have emerged, and 5 previously non-pathogenic organisms have re-emerged in pathogenic form affecting all corners of the globe (L. Feng et al., 2012; Fonkwo, 2008). The recent COVID-19 pandemic also highlights the ongoing danger posed by infectious diseases. When considering the geographical locations of these emerging and re-emerging diseases, it is no surprise to discover that countries in the developing world are the most affected, with immunodeficiency virus (HIV)/acquired immune deficiency syndrome (AIDS), tuberculosis (TB) and malaria having the most significant burden (Fonkwo, 2008). The spread of diseases in under-developed locations are rife due to poor hygiene, poor nutrition, lack of sanitation and clean water and close habitation with animals, providing the perfect breeding ground for infections to spread. As a result, 90% of worldwide infectious disease mortalities are found in the developing world (Gavazzi et al., 2004).

However, wealthier countries also face infectious disease health challenges. For example, in the United States (US), influenza and pneumonia are ranked among the top 10 causes of death (Heron, 2007), accounting for 36,000 deaths annually (Maciosek et al., 2006). Also, despite improvements in influenza and pneumonia rates in the United Kingdom (UK) over the last 2 decades, they still rank in the top 10 causes of death, taking the lives of over 30,000 people annually (Office for National Statistics, 2020).

### *1.2.1 Impact of Influenza*

Influenza is one of the most common infectious diseases, where it is the most prevalent within the ageing population worldwide (Oshitani et al., 2008). Influenza transmission from person to person occurs rapidly in highly congested areas, such as schools and nursing homes, via coughing or sneezing virus-containing droplets. There are four types of seasonal influenza virus, A, B, C and D, where A and B are responsible for seasonal epidemics (Bailey et al., 2018). Influenza A virus subtypes can be classified according to either hemagglutinin or neuraminidase (see *Figure 1* for influenza virus structure), which are the proteins present on their surface, where A(H1N1) and A(H3N2) are the current subtypes circulating in humans. Influenza B viruses do not have subtypes but are instead classified into lineages (e.g., B/Yamagata, B/Victoria, B/Maryland or B/Colorado) currently circulating in humans. The remaining influenza virus types, C and D, are not present in humans or cause mild infections

in humans, so they are not considered when developing vaccines to protect against disease (World Health Organisation, 2018).

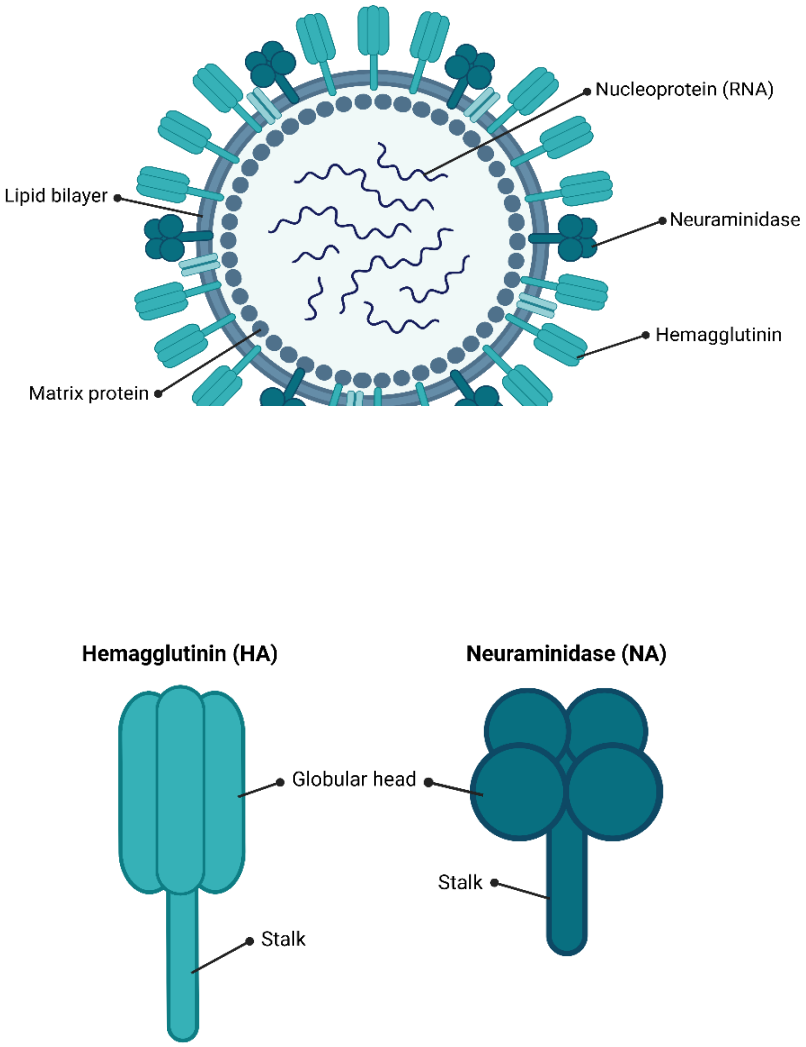


**Figure 1.1: Influenza virus structure** highlighting hemagglutinin and neuraminidase proteins, lipid bilayer, matrix protein and single-stranded RNA. Hemagglutinin and neuraminidase proteins on the surface of the influenza virus are similar in structure, whereby both have a globular head and stalk domain.

Within the UK, there is an enormous economic burden on the NHS due to infectious disease. The occurrence of influenza alone results in hundreds of thousands of GP visits and tens of thousands of hospital stays annually (National Institute for Health and Care Excellence, 2018). Each GP visit is estimated to cost the NHS £30 (National Institute for Health and Care Excellence, 2018), and influenza-related hospital admissions cost the NHS £80 million annually (Oxford Vaccine Group, 2018). During influenza season epidemics, general practitioner clinics and hospitals are often overwhelmed with the high influx of influenza and

its associated illnesses. High influenza-related mortality rates are recorded annually, with average annual rates soaring over 8000, most of which are over 65-year-olds (Public Health England, 2014). For every 1000 cases of influenza, 5.5 general practitioner consultations are reported, costing an estimated £32 per patient visit (National Institute for Health and Care Excellence, 2018). This evidence highlights the need for research in the area of infectious disease to better protect vulnerable members of the population, such as older adults, against influenza. It also highlights the importance of public health and the economic interest to investigate and develop strategies to control and fight the impact of influenza.

## 1.3 Combating Infectious Disease: Vaccinations

Vaccines are considered to be one of the most incredible breakthroughs in modern medicine. The use of vaccinations has been demonstrated to be vital in combating disease, with one infectious diseases, smallpox, being eradicated due to vaccine advancements (Breman et al., 2011). The World Health Organisation estimates that vaccinations prevented at least 10 million deaths worldwide between 2010 and 2015, therefore protecting millions of people from infectious diseases as well as their associated pathological infections, such as pneumonia and diarrhoea (Greenwood, 2014). They also estimated that the administration of vaccinations to older adults reduced the number of hospitalisations by 25-39%, where overall influenza season mortality was reduced by 39-75% (World Health Organisation, 2019). Gross et al. (1995) also reported that the influenza vaccine has been estimated to prevent respiratory illness by 50%, prevent pneumonia by 53%, prevent hospitalisations by 50% and prevent death by 58%. A study in the US assessed the value of the influenza vaccination regarding disease prevention and cost-effectiveness and determined it to be a high-impact, cost-effective method for older adults, further highlighting the value of vaccinations (Maciosek et al., 2006).

The immune system's functionality determines how well an individual responds to vaccination, where there are multiple components involved in mounting an immune response (Clem, 2011). The immune system can be broken down into two broad but interconnecting categories: innate immunity and adaptive immunity. The innate immune response is the immediate reaction to a pathogen entering the body and involves critical immune cells, such as macrophages, neutrophils, mast cells and natural killer cells, that produce a rapid, inflammatory response to destroy a foreign pathogen in the body (Lacy & Stow, 2011). The adaptive immune response can be separated into specialised lymphocytes called B cells and T cells, which display a highly diverse repertoire of antigen-specific recognition receptors. They can specifically identify pathogens via these antigen- specific

receptors, and develop an immunological memory response to pathogens they recognise and eliminate from the body (Schoenborn & Wilson, 2007). B cells are a major component of the adaptive immune response because they produce antibodies specific to a pathogen and are considered to be a crucial element determining whether or not a vaccine is effective (Burton, 2002).

Vaccines work by exploiting immunological memory, which encompasses an essential adaptive immune response component by producing vaccine-specific antibodies. Vaccines can be categorised into two main types: live attenuated and inactivated (Baxter, 2007). Vaccines are often attenuated, which means they are the live but weakened version of a bacteria or virus (e.g., measles, mumps and rubella vaccine). The administered vaccines are an altered version of the virus that is considered to be harmless. Attenuated are the most common and considered to be the most effective. Inactivated vaccines are either whole bacteria or viruses that have been killed or are subunit (e.g., hepatitis A), recombinant protein components of bacteria or viruses, or polysaccharides, which cannot cause disease (e.g., hepatitis B). These fragments can be referred to as antigens. Inactivated vaccines are not as potent as attenuated vaccines and require repeat vaccinations known as boosters (Monto et al., 2009).

Both vaccination types are similar because they are weakened, less harmful versions of bacteria or viruses. Due to the reduced virulence of the virus/bacteria/antigen administered to a patient, the immune system can recognise, destroy and eliminate the virus strain(s) from the body and allows the individual to develop a memory response. This means the required antibody repertoire of the antigen administered in the vaccine is already present in the body and enables an individual to mount a more rapid immune response when they reencounter the particular virus, increasing their chance of protection against infectious disease.

### *1.3.1 Influenza Vaccinations*

One of the biggest challenges faced with controlling infectious diseases is the rapid rate at which pathogens frequently alter their genetic makeup. This is at the heart of why it has proved to be increasingly difficult to control the spread of disease, which has been highlighted in the most recent coronavirus (COVID-19) pandemic (Callaway & Callaway, 2020). The development of vaccinations for influenza has proven to be a challenge each year due to the rapidly evolving strains (Kim et al., 2018). For this reason, the development of influenza vaccines is delayed as much as possible each year to ensure the antigenic and molecular structure is as close as possible to the rapidly evolving virus strains. It is the

structure of the influenza virus genome and the function of its viral proteins that enable antigenic drift and antigenic shift, which facilitates its ability to adapt and evade the immune system (Bouvier & Palese, 2008). The antigenic drift of influenza viruses is a mechanism of variation observed at antibody-binding sites that occurs regularly due to the accumulation of mutations within virus genes. These genetic modifications produce closely related viruses that share similar antigenic properties, allowing the immune system to recognise and respond to these viruses. However, as these subtle mutations increase, these viruses change to evade the immune system (Treanor, 2004). Antigenic drift is commonly observed in influenza type A and B viruses (World Health Organisation, 2019). Antigenic shift is more abrupt and problematic because it forms a new virus subtype (Carrat & Flahault, 2007). This has been particularly challenging for influenza A viruses that result in new hemagglutinin and/or new hemagglutinin and neuraminidase proteins, making the viruses unrecognisable to the immune system (World Health Organisation, 2019).

Ensuring the virus strains within the vaccine are as close as possible to the live virus strains circulating in the population increases the chance of vaccination effectiveness by ensuring the influenza vaccine-specific antibodies produced can recognise and destroy the virus strains when they reencounter them naturally. Virus strain A(H3N2) evolves most rapidly and is considered the most critical in influencing high mortality and morbidity rates of the influenza and is considered to be at the core of significant hospitalisation rates and death (Monto & Petrie, 2019). Despite attempts to reform the vaccine each year, sometimes twice a year, the vaccine effectiveness rate is still alarmingly low for older adults regardless of the similarity of vaccine virus to live circulating virus (World Health Organisation, 2015). The impact on older adults is greatest due to a phenomenon known as immunosenescence, which is the ageing of the immune system resulting in immune dysregulation.

## 1.4 Immunosenescence

Older adults are considered more vulnerable than younger adults to infectious disease due to their sub-optimal, gradually deteriorating immune systems, a phenomenon referred to as immunosenescence (Solana & Pawelec, 2004). The decline in immunity, also referred to as biological ageing, results in the increased risk of cancer, autoimmune and chronic diseases, inadequate vaccination responses and increased susceptibility to infectious diseases, such as influenza (Haq & McElhaney, 2014). Immunosenescence has an influence on both innate and adaptive immunity, whereby in the case of innate immunity, the suppression of natural killer (NK) cells, dendritic cell (DC) and neutrophil activity is highlighted throughout the literature (Solana et al., 2012). The downstream effect of this is a decreased production of cytokines

and lower cellular cytotoxicity. Key elements that influence adaptive immunity include decreased naïve T cell clonal diversity, defective T cell memory and altered T cell signalling (Haq & McElhaney, 2014). This age group experiences thymus involution (i.e., shrinking), which is the atrophy of the thymus resulting in altered structure and function (DeWitt & Luebke, 2015). The thymus is responsible for producing and replacing naïve T cells in the periphery, which is essential for these naïve T cells to trigger immune responses against foreign antigens (Globerson & Effros, 2000). Decreased thymic function, and consequently senescence of naïve T cell production, results in a reduced T cell repertoire, also known as clonal diversity of naïve T cells, and reduced chance of older adults mounting an immune response to a novel foreign antigen (Dewan et al., 2012). Important T cell subsets, CD3+, CD4+, and CD8+, have been observed to decrease with age, further highlighting the decline of T cell function within this population and increasing their susceptibility to infectious disease (Ginaldi et al., 2001). Collectively, this innate and adaptive immunity suppression within the ageing population results in reduced vaccine effectiveness and the inability to deal with persistent infections (Pawelec, 2018). This highlights the necessity to expand on this research area to improve the quality of life of those affected by the disease and reduce the burden of influenza.

## 1.5 Enhancing Vaccination in Older Adults

Improving the responses of the elderly to vaccination is more important than ever due to influenza directly or indirectly contributing to the four leading causes of mortality, with the highest rates observed in older adults. In addition, advances in average life expectancy have predicted that the worldwide elderly population is expected to increase from 600 million to nearly 2 billion by 2050 (Haq & McElhaney, 2014).

Pharmaceutical avenues have been explored to improve vaccination, such as the addition of chemical vaccine adjuvants, in the hope that individuals will present greater immune responses and therefore improve vaccine effectiveness (McKee et al., 2007). The addition of chemical adjuvants aims to stimulate inflammatory immune cells to better aid the activation and presentation of antigen presenting cells (APC) and antibody production. Conventionally, aluminium salt adjuvants have been implemented but have not been effective, which has led to the utilisation of new adjuvants, including emulsions and virosomes that are more effective (Principi & Esposito, 2012). An example of this is the MF59 adjuvanted influenza vaccine, an oil-in-water emulsion, 25% more effective against seasonal influenza in the elderly than its non-adjuvanted comparator (Van Buynder et al., 2013). Various studies have demonstrated the benefits of the addition of MF59 as an influenza vaccine adjuvant, from

enhanced antibody production to improved antibody binding affinity and a more diverse antibody repertoire (J. Yang et al., 2020). The over 65-year-old adjuvanted (MF59) influenza vaccine, in comparison with non-adjuvanted vaccines, was demonstrated to be more effective at reducing influenza-related outcomes, including pneumonia-associated hospitalisations (Domnich et al., 2017). However, despite these improvements in immune responses to vaccination as a result of vaccine adjuvants, overall vaccination responses for this vulnerable population are still poor. An alternative method to vaccine adjuvants that has been explored to improve influenza vaccine immunogenicity in older adults is the administration of the influenza vaccine via different routes. Research has been conducted to administer vaccinations intranasally and intradermally instead of the conventional technique of subcutaneous injection or intramuscularly. However, no significant differences were found between intradermal and intramuscular administration, where intradermal influenza vaccines are no longer recommended (Chi et al., 2010).

Whilst pharmaceutical methods have been shown to improve vaccine efficacy, they have not improved the immune responses of older adults to the same degree as young adults, indicating the need for alternative methods to improve vaccine effectiveness for this vulnerable population (Pereira et al., 2020). One such alternative approach concerns the role of psychological and behavioural factors in modulating vaccine responses. To date, there is a plethora of research to suggest that behavioural and psychological effects, such as stress, physical activity, nutrition, sleep and mood, can act as immune modulators, where they have been observed to alter immunity (Marsland et al., 2006; Pascoe et al., 2014). Of particular interest and relevance for this thesis is the relationship of mood, referred to as negative and positive affect, and the immune system and, consequently, vaccination responses. Specifically, researchers have explored the potential of psychological interventions to alter psychological factors, such as mood and subsequently alter immune parameters and responses to vaccination. The research surrounding this has shown promise and is analysed in-depth in *Chapter 2* of this thesis.

## 1.6 Chapter Summary

In this chapter, the reader was introduced to the global impact of infectious disease, with a particular focus on influenza and its high morbidity and mortality rates, as well as economic implications. The importance of vaccination for combating infectious disease was outlined, as well as the need for alternative avenues for improving vaccination responses in the ageing population. In *Chapter 2* of this thesis, psychological factors capable of modulating immune responses to influenza vaccination are introduced with an in-depth review of this literature.

# 2 Chapter 2: Psychological Influences on Immunity

## 2.1 Chapter Introduction

In *Chapter 1*, the importance of vaccination for the control and eradication of infectious diseases was introduced. Influenza is responsible for significant hospitalisation and mortality rates among older adults due to the natural decline of immune functionality with ageing, known as immunosenescence (Solana & Pawelec, 2004). To date, pharmaceutical avenues have had a limited impact on improving the effectiveness of vaccination for older adult populations (Domnich et al., 2017; McKee et al., 2007). In contrast, behavioural and psychological factors have been demonstrated to act as immune modulators capable of influencing vaccine efficacy. In this chapter, some historical context to this thesis is provided, first by outlining the biopsychosocial model and its role in influencing the emergence of psychoneuroimmunology. The current evidence for the relationships between psychological factors (stress, negative mood and positive mood), immunity and vaccination are then critically reviewed, highlighting current limitations of the literature and knowledge gaps. Finally, an overview of the prior research into psychological interventions as an avenue for altering immunity, with a particular focus on vaccination, is presented and critically analysed.

## 2.2 The Biopsychosocial Model

Historically, the field of medicine has been dominated by the biomedical model, which was the approach used to treat illness exclusively by focusing on the biological basis of pathology. In this biomedical context, the immune system was thought to function as an autonomous system, independent of an individual's behaviour, thoughts, and feelings (Farre & Rapley, 2017). The biomedical model focused on the critical information gained from understanding disease at molecular, cellular and tissue levels and assumed that deviations of these biological variables could fully account for disease. In a now seminal work, George Engel argued that the biomedical model was incomplete and reductionist due to its disregard for important social, psychological and behavioural aspects of health and disease (Engel, 1960, 1977). According to Engel, an alternative approach needed to consider the clinical variability of illnesses, such as diabetes and schizophrenia, by accounting for biological variability and psychological, social, and cultural factors. Additionally, Engel argued that while biological deviations may determine disease characteristics, they do not provide insight into *when* an individual falls ill, further highlighting the importance of gaining further insight into psychological and social variables.

Engel proposed an alternative model, which is described as the biopsychosocial model, drawing on General Systems Theory (Bertalanfy, 1950), which proposes understanding complex systems as comprising of several inter-related events collectively organised into different levels. The biopsychosocial model suggested that an individual's health-related outcomes were not solely dependent on biological factors but could also be influenced by psychological and social-environmental factors. A large body of empirical evidence supported the fundamental ideas of this model. For example, smoking is a significant risk factor in premature mortality from various causes, including cardiovascular disorders, cancer, and chronic pulmonary disease, particularly for lower socioeconomic groups (Jacobs et al., 1999; Lewer et al., 2017). Psychological stress is one of the most widely discussed psychological factors to influence health and has been identified as an important predictive factor in the progression of cardiovascular disease, cancer and HIV (Chida & Vedhara, 2009; Cohen et al., 2007; Dai et al., 2020; Jacobs et al., 1999; Lewer et al., 2017; Moreno-Smith et al., 2010; Steptoe & Kivimäki, 2013). Loneliness and social isolation have also been linked to premature mortality and increased risk of depression (Holt-Lunstad et al., 2015; Tabue Teguo et al., 2016; Tilvis et al., 2011).

The biopsychosocial model played an important role in the progression of clinical care and practical clinical guidelines, whereby more empathy and compassion were brought into medical practices to improve patient diagnosis, health outcomes and patient care (Borell-Carrió et al., 2004). This model highlighted the importance of understanding previously considered “stand-alone” physiological systems, such as the immune system, as being part of a more complex system that is capable of being influenced by behavioural, psychological and social aspects of an individual’s environment.

## 2.3 Psychoneuroimmunology

Around the time Engel proposed the biopsychosocial model, a parallel line of research started to describe the biological pathways that could explain some of the observed relationships between social, psychological, behavioural factors and health. This field of research, now referred to as psychoneuroimmunology (PNI), identified bidirectional relationships between the central nervous and immune systems (Ader & Cohen, 1975). Preliminarily research conducted by Robert Ader and Nicholas Cohen (1975) demonstrated that the behavioural conditioning of rats was capable of eliciting immune modifications. Their early work identified that immunosuppression could be behaviourally conditioned in a study investigating induced taste aversion in rats. In this study, rats were administered an artificial sweetener drink (saccharin) in conjunction with a toxic immunosuppressive agent

(cyclophosphamide), that causes temporary gastrointestinal upset. As a result, rats associated the taste of the saccharin solution with illness, and therefore acquired an aversion to saccharin solution, whereby saccharin consumption was reduced by 61-68%. Researchers found that rats experienced the immunosuppressive effects of cyclophosphamide, even in the absence of cyclophosphamide, when consuming saccharin. This indicated that despite saccharin being safe to consume, it was suppressing the rat immune system due to the previous repeated cyclophosphamide and saccharin exposure. This research was some of the first evidence to suggest the nervous and immune systems may interact and was ground-breaking in influencing research in this field.

The biopsychosocial model, and research such as that of Engel's and his contemporaries inspired a proliferation of research into how psychological experiences and processes could result in biological effects relevant to health. Key to this was the elucidation of pathways by which brain functions, triggered by psychological experiences, was driving change with other physiological systems, such as the immune system. The next section will outline evidence demonstrating the bidirectional relationship between the nervous and immune systems. Following this, research outlining the influence of psychological factors (stress and mood) on immune responses will be outlined, with a particular focus on vaccination.

### *2.3.1 Neuroendocrine and Immune System Integration*

This section will outline some of the evidence that has been accumulated demonstrating biological pathways between the central nervous system (brain & spinal cord) with immunity. The nervous system consists of nerves that transmit impulses of sensation to communicate electrical information via two pathways: central and peripheral nervous systems. The central nervous system consists of the brain and spinal cord and plays an essential role in communicating sensory and motor information to and from different parts of the body. The peripheral nervous system refers to the parts of the nervous system outside of the brain and spinal cord, including the cranial nerves, spinal nerves and their roots and branches, peripheral nerves, and neuromuscular junctions. The peripheral nervous system can be conceptually divided into the autonomic nervous system (ANS) and somatic nervous system. The somatic nervous system works with the central nervous system to communicate sensory and motor signals. The ANS controls the function of organs and glands, where it works in conjunction with the endocrine system to produce hormones (Ader et al., 1995; Novack et al., 2007).

#### *2.3.1.1 Autonomic Nervous System*

Of particular interest in this section is the neuroendocrine and immune system integration observed in the ANS branch of the nervous system, which is further divided into the sympathetic nervous system (SNS) and parasympathetic nervous system (PNS). These systems and integrations with the immune system are described in detail below. The ANS is a component of the peripheral nervous system that regulates involuntary physiologic processes, including heart rate, blood pressure, respiration, digestion, and sexual arousal (Waxenbaum et al., 2022).

Several studies have established that the autonomic nervous system (ANS) plays a critical role in mediating nervous and immune system integration (Elenkov et al., 2000; Ganta et al., 2004; Helwig et al., 2008; Morrison, 2011; Tracey, 2009). Lymphocytes, a key component of the immune system, carry receptors for various hormones (e.g., cortisol), indicating that the cellular interactions that mediate the neuroendocrine environment can modulate humoral and cellular immunity. This is a bidirectional process, whereby the release of molecules by an activated immune response, due to the production of neuropeptides and hormones by lymphocytes, can influence neural and endocrine function (Novack et al., 2007). The bidirectional communication between the branches of the ANS, sympathetic and parasympathetic nervous systems, and the immune system, as well as the relationship between the hypothalamic pituitary adrenal (HPA) axis and stress is described in more detail below.

##### 2.3.1.1.1 Sympathetic Nervous System

A primary function of the SNS is to prepare the body in threatening, psychologically stressful situations by increasing heart rate and blood pressure and inducing pupil dilation and sweating, known as the "fight or flight" response. Sympathetic nerves found in primary and secondary lymphoid organs release various neuropeptides, which can influence immune responses. For example, when subject to acute stress (e.g., getting into an argument with a friend or getting into a car accident), an individual will secrete the neuropeptides epinephrine and norepinephrine from sympathetic nerve endings. These neuropeptides can also be defined as catecholamines because the adrenal glands synthesise them. As a result of acute stress, epinephrine and norepinephrine released into the bloodstream cause increases in blood pressure, heart rate and blood sugar levels. Epinephrine and norepinephrine adrenergic receptors present on immune cells are also capable of altering immune responses (Bucsek et al., 2018). For example, a study found that the activation of adrenergic receptor signalling downregulated CD8+ T cell mediated anti-viral responses to

influenza (Grebe et al., 2009). This study also found that SNS activation hindered APC activation of CD8+ T cells, which was in alignment with previous research that demonstrated dendritic cell downregulation as a result of norepinephrine adrenergic receptor binding (Maestroni, 2002).

In addition to catecholamine signalling, cytokines also play an essential role in the communication between the SNS and immune system. Cytokines are signalling proteins synthesised and secreted by a range of immune cells, including monocytes , lymphocytes, neurons and endothelial cells (cells that form the barrier between blood vessels and tissues) (Galic et al., 2012). SNS activation has been demonstrated to have a direct impact on pro-inflammatory immune activity due to the release of a range of pro-inflammatory cytokines (IL-1β, IL-6, IL-10, IFNγ and TNF-α), as a result of adrenergic receptor binding on the surface of lymphocytes (B cell and T cells) and monocytes (macrophages and dendritic cells) (Pongratz & Straub, 2014). For example, catecholamine binding to adrenergic receptors on dendritic cells was shown to modulate cytokine (decreases IL-12, increases IL-10) production and inhibit migration and T helper cell priming (Maestroni, 2002; Maestroni & Mazzola, 2003; Seiffert et al., 2002). Similarly, adrenergic receptor binding of catecholamines on dendritic cells were also demonstrated to induce a pro-inflammatory IL-17 immune response due to altered T helper cell priming (Manni et al., 2011). TNF-α regulation has also been shown to be influenced by norepinephrine binding to macrophages, further highlighting the communication between the SNS and inflammatory immune response (Spengler et al., 1990).

As mentioned previously, the communication between the SNS and immune system is bidirectional. Therefore, in response to a pathogen entering the body, immune system activation will, in turn, produce pro-inflammatory mediators, such as cytokines. If the threshold of pro-inflammatory cytokines is high enough, SNS activation occurs. For example, IL-1β, released by activated immune cells (predominantly stimulated monocytes), was identified as being a critical mediator of SNS activation via hepatic vagus nerve binding to result in paraganglia (collection of cells near the adrenal glands that release epinephrine and norepinephrine) activation (Goehler et al., 1997).

#### 2.3.1.1.2 Parasympathetic Nervous System

Another pathway by which the immune system is impacted by the nervous system is the PNS. The PNS comprises the vagus nerve and its branches distributed throughout the body in multiple organ systems, and it is critical for communicating sensory information (DuBois &

Foley, 1936). The PNS has multiple roles, including restoring the body's equilibrium after encountering acute stress via hormone secretion, known as homeostasis. The hypothalamus, attached to the pituitary gland, controls the release of hormones (glucocorticoids) from the pituitary gland to control this balance (M. R. Irwin & Slavich, 2016; E. M. Sternberg, 2006). For example, after an individual has experienced acute stress, the PNS is activated due to the presence of hormones (epinephrine and norepinephrine) in the bloodstream, which in turn activate the hypothalamus to release glucocorticoids from the pituitary gland.

The PNS plays a key role in regulating cytokine production to ensure inflammatory immune responses, such as the upregulation of TNF-α, via vagus nerve activity are balanced. This is important because chronic immune inflammation has been associated with a variety of diseases, including cardiovascular disease, rheumatoid arthritis, atopic dermatitis and psoriasis (Chovatiya & Silverberg, 2019; Lopez-Candales et al., 2017). Immune modulation is observed via vagus nerve ending neurotransmitter acetylcholine interactions with macrophages, resulting in the inhibition of macrophage activity and consequently inhibited pro-inflammatory cytokine release (Tracey, 2007). Vagus nerve activation has also been demonstrated to inhibit the release of pro-inflammatory cytokines, such as IL-1 and IL-6, associated with coronary heart disease (Janszky et al., 2004). Therefore, the PNS is key for regulating cytokine production to ensure immune inflammation is controlled and further highlights the integration of the nervous and immune systems.

#### *2.3.1.2 Hypothalamic Pituitary Adrenal Axis*

The hypothalamic pituitary adrenal (HPA) axis is another key pathway mediating physiological systems in the body and has been studied extensively. It is also a major component of the homeostatic response that mediates the effects of stressors by regulating physiological processes, such as metabolism, immune responses and the ANS. It is a complex system involving the hypothalamus, anterior pituitary gland and adrenal gland. When encountering stressful situations, whether that be acute stress (brief and time limited stress, often no more than a few weeks in duration, e.g., witnessing a crime) or chronic stress (persistent stress over time with an unknown endpoint, e.g., bereavement, stressful work environment) the HPA axis is activated via the release of corticotropin releasing factor (CRF) from the hypothalamus, which stimulates the release of adrenocorticotrophin (ACTH) from the pituitary gland (S. M. Smith & Vale, 2006). This subsequently leads to ACTH mediated stimulation of the adrenal glands that produce and secrete glucocorticoids, most notably cortisol. This steroid hormone, cortisol, has been demonstrated to have detrimental

immunological effects and long-term effects on health when present in abundance (Dedovic et al., 2009; Esther M. Sternberg et al., 1992).

PNI researchers have also investigated psychological stress and its brain physiology modifications via experimental functional magnetic resonance imaging (fMRI), which lead to altered immune function (Dedovic et al., 2009). For example, a study showed that participants engaging in a timed mental arithmetic task designed to induce moderate acute stress demonstrated increased activation of specific regions of the brain measured by fMRI and increased salivary cortisol production (Dedovic et al., 2005). Stress PNI studies in humans have pinpointed the activation of the HPA axis as one of the key physiological markers of stress, which is often used as a reference for measuring stress-induced physiological modifications (Dedovic et al., 2005).

The duration of stressor exposure, and subsequently cortisol levels as a result of HPA activation, has been shown to influence the immune system differently. For example, acute stress responses in young, healthy adults typically do not impose a health burden, and therefore do not result in long-term damage to health (Schneiderman et al., 2005). Acute bursts of stress are even observed to enhance immune cell function because of the activation of the sympathetic nervous system, which leads to an increase in immune cell circulation from lymphatic organs (F. Dhabhar, 2008; Elenkov et al., 2000). Research by Dhabhar (2000) illustrated that the administration of acute stress hormones, such as corticosterone (i.e., cortisol equivalent found in rodents), to rats or mice immediately before antigen exposure significantly enhanced skin delayed-type hypersensitivity (DTH) reactions. This was found to be a result of acute stress significantly increasing the mobilisation of leucocytes from the blood to the skin, therefore highlighting the immune enhancing effects of acute stress on cellular immunity (F. S. Dhabhar, 2000). Further research by Dhabhar et al. (2000) found that IFNγ receptor knockout mice failed to show acute stress-induced enhancement of skin DTH, which suggested that the inflammatory response induced via IFNγ is an important mediator of immune enhancement in the context of acute stress (F. S. Dhabhar et al., 2000). This evidence highlights the beneficial effects of acute stress activation of the HPA axis to result in the release of hormones, such as cortisol, to increase inflammation via IFNγ and leucocyte mobilisation.

In contrast, in the case of chronic stress, individuals are subject to stress-induced changes for a prolonged period of time, which makes it too challenging for their physiology to maintain homeostasis. This process is encapsulated by the term Allostatic Load and is

considered to be a key mechanism by which individuals are more susceptible to infectious disease when chronically stressed (McEwen, 1998). Types of allostatic load include frequent and prolonged activation of physiological systems, such as the HPA axis, and failure to shut them off after stress. Individuals who experience chronic stress, that is stress over an extended period of weeks or months and often with no defined endpoint, have been shown to experience downregulated immune responses, whereby chronic stress is capable of eliciting long-term effects on health (Cohen et al., 2007; Nes & Segerstrom, 2017; Segerstrom et al., 2012). Chronic stress, which manifests in the physiological response of excess HPA axis activation and cortisol release, has been shown to result in greater susceptibility to infectious disease, delayed wound healing and decreased antibody production to vaccination (Glaser & Kiecolt-Glaser, 2005; Sacadura-Leite et al., 2014; S. M. Smith & Vale, 2006; Vedhara et al., 1999). Chronic HPA axis activation, and therefore chronic glucocorticoid secretion, has been shown to affect hippocampal function (part of the brain that plays a major role in learning and memory) (Raber, 1998). In the brain, the hippocampus has been noted to have the highest concentration of glucocorticoid receptors, whereby chronic stress has been shown to result in the atrophy of hippocampus neurons. Consequently, this research has found that chronic HPA axis activation has been implicated in the development of cognitive defects (Karin et al., 2020). From an immunological perspective, chronic levels of glucocorticoids, capable of binding to the receptors of immune cells (Eskandari & Sternberg, 2002; Marques-Deak et al., 2005), were found to promote Treg differentiation (key suppressors of the immune system) and reduce the levels of circulating T cells (Tischner & Reichardt, 2007). Most notably are Th1 and Th17 cells that are important for infection protection via their secretion of pro-inflammatory cytokines (e.g., IFNγ, IL-6) (Sheng et al., 2021; Wrona, 2006). Therefore, the repression of these T helper subtypes increases the risk of infection for chronically stressed individuals (Franchimont, 2004). Evidence of the influence of acute and chronic stress on immunity and vaccination is described in detail in *Section 2.5.1* (*Influence of Negative Emotional Experience on Influenza Vaccination*).

## 2.4 Evidence for Psychological Influences on Infectious Diseases

The increasing recognition of a bidirectional relationship between the nervous system and immune system, outlined above, and the development of the biopsychosocial model led to the expansion of PNI research. The foundation of the research in this area is based on the premise that psychology, behaviour and physiology interact to influence health outcomes including, but not limited to, cancer (Ramirez et al., 1989; Seifter et al., 1973), wound healing (Emery et al., 2005) and asthma (Castés et al., 1999). Of particular relevance for this thesis is

the body of PNI research that has focused on infectious diseases. The expansion of the field of PNI research has highlighted a plethora of behavioural (e.g., sleep, exercise, nutrition) and psychological (e.g., stress, positive and negative mood) factors that have been shown to elicit physiological changes in the body to result in altered immune function and altered responses to infection and vaccination (Cohen et al., 2001; Grant et al., 2008; Kiecolt-Glaser et al., 1996; Prather et al., 2012). Of these factors, stress, positive affect and negative affect, and their influence on vaccination, are the main focus of this thesis. The literature on these factors and their influence on immune responses and vaccination will be outlined in detail in the remainder of this chapter. Research investigating the relationship between psychological influences on the immune system and vulnerability to infectious disease have primarily utilised three approaches: observational studies, viral challenge studies and vaccine studies. Here, evidence from each of these approaches will be examined in turn.

### *2.4.1 Observational Studies*

Early PNI research provided observational evidence that increased stress and depression were associated with a greater incidence of self-reported respiratory infections (Glaser et al., 1987; McClelland et al., 1980; Parens et al., 1966; Sarason et al., 1985). In some cases, this research has focused on experiences of life stressors and linked these with self-reporting of infection symptoms. For example, Sarason et al. (1985) prospectively followed 163 submarine school students for a year and found that negative life events, measured via a Life Experiences Survey that asked participants to self-report events they have experienced during the last year, were correlated with increased self-report of illness symptoms over the year (ranging from respiratory infection to ankle pain to skin disorders).

Other research has opportunistically sought to examine infection susceptibility during stressful periods. For example, Glaser et al. (1987), in a study of 40 medical students in their first year of training, reported a greater incidence of self-report symptoms of infectious illness during examination periods compared to non-examination periods. Further, researchers obtained blood samples at first, third and fifth examination periods and one month before each to measure the effect of academic stress on the control of latent Epstein Barr virus (EBV) by antibody titres, as well as IFNy production by stimulated lymphocytes. The data showed that EBV antibody titres were elevated during examination periods, suggesting reactivation of latent EBV, and IFNy levels were significantly decreased, indicating poorer cellular control of latent EBV. This study went a step further than antibody and cytokine measures to also investigate stress-related changes of specific memory T lymphocytes to recognise and lyse EBV antigens on the surfaces of cells. Consistent with the

notion of stress induced down-regulation of immune regulation, there was a significant decrease in cell killing during examination periods. However, due to limited blood sample collection this was only conducted on a small proportion (*n*= 4) of the student population. This study supports the linkage between stress and immunosuppression, as well as the relationship between stress and increased self-report of illness symptoms. Another study conducted by Parens et al. (1966) investigated first-year nursing students and their adjustment to being separated from home after 6 weeks. This study found that students who adjusted poorly at the 6-week mark, with high depressive effects (measured using four questionnaires: Depression Inventory Scale Adjustment Scale, Past Object Loss Scale and The Beck Inventory), reported illness more frequently during the remainder of the school year (Parens et al., 1966).

Together, while the above cited studies provide evidence of an association between psychological factors and susceptibility to infection, most are limited by their reliance on self-report of infection symptoms. These self-reports are subject to biases, most notably that psychological distress is also associated with increased symptom perception(Cohen et al., 1995; Salovey & Birnbaum, 1989). For example, Salovey and Birnbaum (1989) conducted experiments to assess the effects of mood on symptom appraisal and found that individuals who experienced sadness reported more aches and pains and greater discomfort than happy individuals. Beyond the issue of increased symptom perception, the above observational studies do not account for the fact that some individuals exposed to infection may not display symptoms or may only have mild symptoms, despite serological testing, such as antibodies, indicating infection. For example, this has been evident throughout the COVID-19 pandemic, whereby recent research has found that 40.5% of the population with confirmed COVID-19 were asymptomatic (Ma et al., 2021). The absence of serological testing to verify infection is an important limitation of many observational studies investigating the relationship between psychological factors and infectious diseases.

However, some studies have examined the association between psychological and behavioural factors and infection susceptibility with serologically verified outcomes (e.g., upper-respiratory infections) (Boyce et al., 1977; Graham et al., 1986; Meyer & Haggerty, 1962). For example, Graham et al. (1986) prospectively studied 235 adults to investigate the relationship between stress and upper respiratory tract infection and found that highly stressed individuals had a greater number of verified illness episodes and self-report symptom days. Further research has also examined latent viruses (e.g., herpes simplex virus (HSV)) , which is a type of virus that lies dormant within in a cell and can be reactivated to

cause persistent infection. (Longo & Koehn, 1993; VanderPlate et al., 1988). VanderPlate et al. (1988) found there was a positive association between stress and HRV recurrences for duration of disease that lasted less than 4 years.

The evidence above highlights the importance of psychological factors on infection incidence for both symptomatic and serologically verified studies. However, these studies were observational and therefore could not suggest causal relationships between the two. In many of these studies it is challenging to ascertain whether stress led to the illness susceptibility or if those more susceptible to illness ended up with higher stress, or poorer mood. Additionally, these studies do not control for varying exposure levels meaning there is the possibility that those with higher levels of stress, such as healthcare workers, are more likely to receive greater infection exposure and are therefore also likely to experience a higher incidence of infection.

### *2.4.2 Live Virus Challenge Studies*

Studies providing better evidence for the causal relationships between psychological factors and serologically verified infection comes from live virus challenge studies. In the case of live virus challenge studies, participants are deliberately exposed to a known dose of a pathogen (e.g., corona and rhinoviruses) and rigorously monitored, to measure infection susceptibility and severity via antibody production and symptom observations. The advantage of viral challenge studies is that the initial exposure to the infectious agent is carefully controlled, which is something that is not possible in more naturalistic observational studies of infection. Another strength of these studies is that they measure both antibody measures and symptoms to consider participants who present antibodies to the dose of the pathogen but do not display symptoms and provides some insight into asymptomatic illness. This is also important because individuals can produce antibodies prior to them displaying symptoms (Patrozou & Mermel, 2009). Live viral challenge studies allow for participants to be quarantined and allows for regular serological testing for infection. They also provide researchers with helpful exposure responses in controlled quarantined conditions to eliminate and standardise confounding factors, such as environment and behavioural influences (e.g., diet, sleep, exercise). This gives researchers the confidence to be sure the relationships between psychological factors and infection are causal rather than correlational.

A number of large viral challenge studies have examined the relationship between psychological factors and infection susceptibility (Broadbent et al., 1984; Cohen et al., 1991,

1993, 1998; Totman et al., 1980). Types of psychological influences can vary, whereby some studies investigated life event stressors and others have focused on chronic stress, but have consistently supported a causal relationship between psychological influences and viral infection. For example, one viral challenge study sought to investigate the influence of recent life stressors on susceptibility to infection and found that social losses (e.g., death of a family member) and social deprivation (e.g., poverty) were significant life stressors associated with a higher risk for more severe illness (Totman et al., 1980). An individual's perception of psychological stress has also been considered in viral challenge studies. Cohen et al. (1991) conducted one of the first studies to investigate whether psychological stress could suppress host resistance to infection. Healthy subjects were quarantined and exposed to rhinovirus (type 2, 9, 14 or respiratory syncytial) or coronavirus (type 229E) and monitored for evidence of infection via virus-specific antibody measures and symptoms. Researchers found that both respiratory infection and clinical colds increased in a dose-response manner with increases in psychological stress. These findings were not altered when personality variables (e.g., self-esteem) and other lifestyle effects (e.g., smoking, alcohol consumption, sleep) were controlled for, suggesting a causal relationship between psychological stress and suppression of infection resistance. In a later study, Cohen et al. (1993) explored a combination of these psychological influences by investigating stressful life events, perceived stress and negative affect and their influence on susceptibility to a common cold virus. Psychological stress was shown to increase susceptibility to infection, which is consistent with the previous study. Researchers also found that high scores for all three stress scales were associated with a greater risk of developing a cold.

Social support, which is strongly entwined with psychological wellbeing, has also been the subject of viral challenge study research. In one such study, participants were asked to provide self-report details on their social network, social support and complete measures of positive and negative moods (Cohen et al., 2003a). Participants were then quarantined and exposed to one of two types of rhinoviruses. Pre-exposure antibodies, 4-week post-infection antibodies and saliva cortisol levels were measured, alongside self-reported cold symptoms. Researchers found that positive mood was associated with decreased susceptibility to infection, and sociability (i.e., social support) was also associated with increased positive mood and decreased infection risk. They also found increased self-report of illness symptoms and high levels of virus-specific antibodies in low sociability groups experiencing depressive affects. Interestingly, this study also indicated volunteers self-reported illness to be more severe than the study's objective measures of illness, which suggested that self-reported

illness was not necessarily related to antibody measures and highlighted the importance of measuring both. In particular, the importance of collecting objective measures, such as antibodies, for infection studies instead of relying solely on self-report data. This study was also conducted over a broad age range (18- to 54-year-olds) and found that being a younger adult was associated with fewer colds than being older, which aligns with the evidence in *Chapter 1* of this thesis outlining immune system downregulation with ageing (immunosenescence).

Collectively, these live viral challenge studies have demonstrated that psychological factors (e.g., stress and depression) can increase susceptibility to infection at a clinically meaningful level. However, the high level of control of live viral challenge studies can also be a limitation because these studies do not accurately represent real-life environments. There are also ethical concerns when conducting challenge studies with older adults, who have a considerably higher risk of morbidity due to their compromised immune systems and increased vulnerability to adverse reactions to pathogen exposure. As such, there is a paucity of research in older adults. There are also ethical concerns when conducting challenge studies with particularly dangerous infectious diseases, such as human immunodeficiency virus (HIV). When untreated, HIV can lead to acquired immunodeficiency syndrome (AIDS), which currently has no effective cure. As a result of these limitations, these studies tend to focus on healthy young adult populations and are restricted to investigating viruses that are not considered to be life threatening (e.g., common cold viruses). This also means that few studies are done on vulnerable populations, such as older adults.

### *2.4.3 Vaccination Studies*

Given these limitations, researchers investigating the association of psychological factors and infection in older adult populations have also turned to vaccination studies as an alternative approach to examine the relationships in at risk populations such as older adults (Kiecolt-Glaser et al., 1996; Vedhara et al., 1999). In both vaccination and live virus studies, the immune system is activated in the same manner by recognising a foreign antigen in the body and producing antigen-specific antibodies. Vaccination administration studies are developed to induce an immune response to produce vaccine-specific immune cells to protect an individual from infection. These studies are not conducted in quarantine, which means it more accurately reflects real-life and confounding factor exposure. Yet, dose exposure remains controlled. A further benefit of vaccination studies, specifically those focusing on influenza and COVID-19, is that researchers can take advantage of routine vaccination care programs and, therefore, increase study recruitment and recruit more representative

samples. Additionally, as outlined in *Chapter 1*, older adults often display poor vaccination responses due to immunosenescence, which makes studying how psychological factors are capable of influencing vaccination responses clinically relevant.

Given the utility of the vaccination paradigm to examine psychological influences on immunity, a large corpus of vaccination studies now exists, covering a range of vaccines (e.g., hepatitis, tetanus, pneumococcal) and populations. Here, given the focus of this thesis, the discussion below will primarily restrict its focus to influenza vaccination studies conducted in older adult populations, which provide insight into the immune influence of psychological factors (specifically measures such as stress, negative and positive emotional experience). However, evidence from other populations and other designs will be considered where there is a shortage of evidence on older adults and influenza vaccines.

## 2.5 Psychological Influences on Vaccination Responses

It is important to address what it meant when referring to affect in this thesis. Affect, in the psychological literature, is often used as an umbrella term to refer to any experience of feeling, emotion or mood. Positive affect reflects the extent to which a person feels enthusiastic, active and alert, whereby a high positive affect is a state of high energy, full concentration and pleasurable engagement and low positive affect is characterised by lethargy (Watson, Clark, & Tellegen, 1988). Negative affect refers to unpleasurable engagement that encapsulates a variety of mood states including anger, fear, distress, depression and hostility. Researchers do not typically consider stress to fall under the umbrella term of negative affect, as demonstrated by the fact that the two are measured differently. However, overall negative emotional experiences encompass both stress and affect. For this reason, stress and affect will be discussed separately within this thesis, however important studies showing evidence for their interaction with each other will be mentioned where relevant.

Affect measured over a period of weeks or months is often referred to as trait or dispositional affect, and affect measured in the present moment or over the last day or week is referred to as state affect (Riepl et al., 2016). Both state and trait affect are most frequently measured using validated self-report questionnaires. The Positive and Negative Affect Schedule (PANAS) is one of the most widely used methods of measuring affect, where it has been extensively used for over 20 years for a range of research from anxiety and depression (Watson, Clark, & Carey, 1988) to cigarette consumption (Becoña et al., 1998). There are, however, a range of alternative self-report questionnaires including the Scale of Positive and

Negative Experience (SPANE) (Diener et al., 2009), the Dynamic Visual Analogue Mood Scales (DVAMS) (Barrows & Thomas, 2016) and the Affective Slider scales (Betella & Verschure, 2016). These affect measures are described in more detail later in this thesis (see *Chapter 3*). The relationship between positive and negative affect is somewhat debated, whereby some researchers argue that positive and negative trait affect are independent constructs (Schmukle et al., 2002; Watson, Clark, & Carey, 1988; Watson & Clark, 1997). Others argue they are opposite ends of a continuum, given significant negative correlations are often observed when measuring both positive affect and negative affect (Schmukle et al., 2002). While there is no explicit consensus, researchers interested in affect often characterise positive and negative affect as being quasi-independent, given the evidence that people can concurrently experience high levels of positive and negative affect (Larsen & McGraw, 2011). As such in this thesis, positive and negative affect are conceptualised as different, if related, measures of emotional experience, as opposed to being opposite ends of a single continuum (Remington et al., 2000). Like stress, as was discussed briefly in the earlier sections of this chapter, chronic and acute experiences of affect have been evidenced as differentially influencing the immune system. Research demonstrated it was possible to induce positive and negative affect for study purposes to investigate their relationship with the immune system. Similar to the research into stress and immunological changes, studies investigating induced positive and negative affect have observed causal relationships between affect alterations and immunological parameters, including salivary IgA productions, natural killer cell activity, and pro-inflammatory cytokines (IL-2, IL-3, IL-6 and TNF-α) (Pressman & Cohen, 2005). This section aims to introduce the reader to some of the evidence and how, in turn, negative and positive emotional experiences may influence immune responses to influenza vaccination.

### *2.5.1 Influence of Negative Emotional Experience on the Immune System*

In this section, the literature that investigates the influence of negative emotional experience on vaccination responses in older adults will be reviewed, with some consideration given to general immune responses and younger populations. As briefly mentioned above, negative emotional experiences encapsulates both stress and negative affect, whereby negative affect can consist of depression and anxiety. Whilst negative affect is closely linked to the concept of depression, in that low positive affect and high negative affect (both state and trait) are major distinguishing features of depression and anxiety diagnoses, respectively (Teilegen, 2019), this does not suggest that these terms are interchangeable.

#### *2.5.1.1 Negative Affect and Immunity*

Before examining the literature on vaccination specifically, there is a much broader body of evidence concerning the relationship between negative affect and the immune system, as well as with health outcomes more generally that is worth highlighting. Greater negative affect has been shown to be associated with altered immune responses and overall health including an increased risk of physical disorder and mortality, whereby mortality is more evident in depressed older adult populations (Murphy et al., 1988). High levels of trait negative affect have been linked to the development and/or progression of heart disease (Donker, 2000), cancer (Reiche et al., 2005), arthritis (Kiecolt-Glaser et al., 2002), diabetes (Carnethon et al., 2003), and the common cold (Cohen et al., 1998; Consedine & Moskowitz, 2007). Immunological differences have been demonstrated between individuals with major depression disorder (which, in part, is characterised by high levels of negative affect and low positive affect) and those without. For example, major depression has been linked to increased pro-inflammatory immune cell production, such as cytokines IL-1 and IL-6, which play a role in suppressing natural killer (NK) cell activity (Herbert & Cohen, 1993). A meta-analysis conducted by Zorilla et al. (2001) showed that there were nearly forty immunological parameter differences between individuals with a diagnosis of major depressive disorder and those without. Some notable immunological differences observed in individuals with major depression included a reduction in NK cell and T cell counts and functionality, increased CD4+/CD8+ ratios and overall leucocytosis, which was characterised by increased levels of leukocytes in the blood and could be associated with diseases of the bone marrow. In this review, elevated circulating IL-6 levels were observed in individuals with major depression, which has been identified as an immunological biomarker for chronically stressed individuals (Doyle et al., 2006; Mastorakos & Ilias, 2006; Robles et al., 2005; Segerstrom et al., 2012; Tanaka et al., 2014). IL-6 is a pro-inflammatory cytokine that has been shown to increase in response to stress to result in detrimental immunological effects. The pleiotropic nature of IL-6 means it has been implicated in the onset of various diseases, particularly autoimmune diseases (e.g., rheumatoid arthritis) (Tanaka et al., 2014). The role of IL-6 on the immune system and overall health is reviewed in more detail in the next section (see *Section 2.5.1.3*).

Other research has shown the clinical significance of these immunological variations on health, where, for example, reduced NK cell cytotoxicity was associated with increased morbidity for many diseases, including cancer and infectious disease (Whiteside et al., 1990). It is well-established that NK cells are responsible for connecting the innate and adaptive immune systems, where they have a cytotoxic role via the secretion of inflammatory

cytokines (similar to Th1, Th2 and Th17 subsets) but can also be regulatory by suppressing antigen-specific T cell responses via the secretion of IL-10 (Deniz et al., 2008). Similar to IL-6, NK cell cytotoxicity and NK cell derived cytokines have been highlighted to contribute towards the pathogenesis of autoimmune diseases, such as ankylosing spondylitis and psoriasis (Kucuksezer et al., 2021). Research by Maes et al. (1994) found that blunted NK cell activity was a hallmark for major depression. In this study, the severity of depression was measured using The Hamilton Depression Rating Scale (HDRS) and blood samples were obtained to assess NK cells, leucocytes, monocytes and neutrophils. Participants with a history of psychiatric illness (e.g., schizophrenia) or major medical illness (e.g., diabetes) were excluded from taking part. This research found a significant negative correlation between severity of depression and NK cell activity (Maes et al., 1994). Additionally, there was a significant and negative correlation between NK cell activity and the number of leucocytes, monocytes and neutrophils. This highlights that the immunosuppressive effects of major depression can go beyond cytokines and NK cells by also influencing lymphocyte proliferation. The consequences of this have been outlined by Murasko et al. (1987) who found that decreased lymphocyte proliferation was associated with increased numbers of hospitalisations and mortality in older adults. Together, this evidence highlights the role of the dysregulated immune system in individuals with major depression and the possible associated health consequences.

Negative affect has also been implicated in the downregulation of T-cell responses. For example, a longitudinal study conducted to investigate the association between depressive symptoms and cellular immune response in older adults and found that older adults with seven or more symptoms of depression displayed decreased CD4+ T cells compared with older adults who had fewer than seven symptoms (Fortes et al., 2003). This was consistent with other research in young adults, which has shown that depressed individuals showed lower percentages of activated T cells than those who were not depressed (Schleifer et al., 1996). Antibody responses to vaccination provides a measure of competence of the immune system to respond to a foreign antigen, which is why it is so commonly referred to in the literature. However, the impact of T cell production and activation is vital for aiding antibody production, especially in the context of vaccination. An important function of T cells is to activate B cells to produce antibodies specific to a foreign antigen, in this case via vaccination, which means that B cell production of antibodies is dependent on the activation of specific T cell populations (Ahmed & Gray, 1996). The means that the influence of chronic negative

affect, such as major depression, downregulating T cell responses plays an important role in influencing antibody responses to vaccination.

#### *2.5.1.2 Negative Affect and Vaccination*

Multiple studies have demonstrated relationships between negative affect and/or depression with antibody responses to different vaccinations. One of the most studied has been Hepatitis B vaccinations, typically in the context of medical students. Research has examined the relationship between depressive symptoms and vaccination and found that depression is closely related with antibody response following hepatitis B (HBV) vaccination, whereby antibody responses were negatively associated with self-reported depression scores (Afsar et al., 2009). Individuals identified as having seroconverted (defined as ELISA antibody titres ≥10 IU/L after the full course of vaccination) scored highly in most of the medical outcomes short form (SF-36) and low in the Beck Depression Inventory, further demonstrating the relationship of depression on antibody responses and health. Interestingly, physical activity and physical well-being were positively associated with seroconversion, whereby both of these factors were shown to improve the psychological status of highly depressed individuals. Physical activity was also shown to improve mood and in turn immune response activation by increasing T cell counts and upregulating antibody production (Drela et al., 2004). This evidence suggested that increased physical activity may help to improve immunity and vaccination responses of depressed patients. Marsland et al. (2001) investigated a cohort of healthy graduate students over a standard course of 3 hepatitis B vaccinations. To ensure participants were not exposed to prior hepatitis B, via infection or prior vaccination, pre-vaccination antibody levels were assessed, with no graduate students indicated prior exposure. This study found that trait negative affect was capable of influencing antibody responses to hepatitis B vaccination in healthy young adults. Researchers reported participants with higher levels of trait affect to have lower secondary antibody responses to hepatitis B vaccination, which was consistent with prior research by Glaser et al. (1992) that observed individuals who characteristically show high negative affect in response to examination stress mounted delayed primary antibody responses to hepatitis B vaccination. This study was very similar to the one conducted by Marsland et al. (2001) and examined healthy medical students. The study by Marsland et al. (2001) also showed a reduction in stimulated T cell proliferation and alterations in circulating lymphocyte populations, including increased T suppressor and NK cell numbers and reduced B cell populations. Unsurprisingly, a reduction in T cell proliferation was greatest in participants who mounted lower antibody responses to vaccination compared with

individuals who displayed higher antibody responses. This is most likely due to a decrease in the number of T helper cells that are critical for activation of B cell production of antibodies. In contrast, high T helper cell counts were observed in participants with high antibody responses.

Both Glaser et al. (1992) and Marsland et al. (2001) demonstrated that the magnitude of immune response to hepatitis B vaccination was closely related to trait negative affect, which is strong evidence to support the relationship between the two. However, there have been studies that have showed no association between negative affect and vaccine responses (Ayling et al., 2019; Moynihan et al., 2004). For example, Ayling et al. (2019) conducted an observational study to assess a range of psychological factors and their influence on influenza vaccination responses in older adults. In this study, negative affect was not found to be a predictor of vaccination responses. This cohort of older adults is notably different to the student and recent graduate cohorts seen in the Marsland et al. (2001) and Glaser et al. (1992) studies and could be an explanation for the observed differences. It is likely the student and graduate cohorts, particularly around examination times, would have reported higher negative affect compared with the older adult population and therefore meant it was challenging for Ayling et al. (2019) to adequately explore whether negative affect had detrimental effects to vaccination responses. This was evidenced by the non-normal distribution of negative affect scores that were skewed towards having low levels of negative affect. There is also a chance that the nature of the older adult age group and vaccination type (i.e., the influenza vaccination is administered annually compared with hepatitis B that is only administered as one complete schedule of 3 vaccinations) means it is also very likely that the participants in the Ayling et al. (2019) would have had prior vaccination exposure, which was an aspect controlled for in the Marsland et al. (2001) study. This makes it more challenging to assess vaccination responses, and is another possible explanation for the observed differences between these studies.

The evidence presented above described the relationship between trait negative affect, immunity and vaccination responses. Research has also suggested that negative state affect, defined as negative affect experienced in the present moment, is also capable of altering immunity and vaccination responses (Stone et al., 1987, 1994). Daily diary focused studies have shown that daily fluctuations of mood and daily stressful events are capable of altering secretory immunoglobulin A (IgA) antibodies. In one study, subjects were identified as low IgA antibody responders on high negative mood days and high IgA antibody responders on days with high positive mood (Stone et al., 1987). Undesirable work events and undesirable

leisure and household events were also strongly related to IgA levels, whereby day-to-day positive and negative affect were positively and negatively associated with IgA production, respectively. Daily fluctuations in mood that alter antibody production have also been suggested to influence the onset of symptoms of respiratory illness (Stone et al., 1993). Stone et al. (1993) found that a significant elevation in the number of undesirable events found in the 3-to-5-day period prior to the start of illness symptoms, whereby this timeframe is due to a lag between exposure to displaying symptoms. One possible explanation for the influence of stress on immunity could be due to the release of immune suppressing hormones, such as cortisol (described in more detail in the next section). Whilst this research is not definitive, it does demonstrate that daily events and stress can mediate antibody production and increases the likelihood of encountered viruses entering the body to cause infection. This research suggested that the influence of state negative affect on the immune system was consistent with the findings observed for trait negative affect, whereby both have immunosuppressive effects. An interesting outcome presented amongst this research was the importance of desirable events for predicting IgA antibody production, which was in contrast to the majority of stress literature that typically focused on the importance of negative life events, and may even serve as a stronger predictor of secretory antibody compared with undesirable events (Stone et al., 1994). The impact of positive mood on the immune system and vaccination responses will be reviewed later in this chapter (see *Section 2.5.2*).

There is also evidence showing inducing negative affect alters immunity and vaccination responses. For example, Rosenkranz et al. (2003) induced negative affect using an autobiographical writing task to measure alterations in the brain and immune system. Electroencephalography (EEG) (recording of brain activity) and affect-modulated eye-blink startle was used to measure trait and state negative affect, and antibody response to influenza vaccination was assessed to investigate immune responses. Overall, physiological indicators of negative affect (greater relative right-prefrontal EEG activation at baseline, greater relative right-prefrontal EEG activation and larger relative eye-blind startle magnitude in response to negative affect induction) predicted weaker antibody responses to influenza vaccination. This study provided evidence for the relationship between negative affect and the nervous and immune systems. Although this study was unable to determine the specific pathways responsible for these interactions, there is evidence of hypothalamic pituitary adrenal (HPA) axis, hippocampus, and amygdala modulation in response to negative affect induction (Roszman et al., 1985), making the HPA axis a plausible mechanism.

This literature in this section has provided evidence for an association between trait and state negative affect, cellular immunity and antibody responses to vaccination. It has highlighted that individuals with high negative emotional experience may mount less protective immune responses, which could indicate the potential clinical significance of negative emotional experiences for predicting antibody responses to vaccination and susceptibility to disease. Stress is the most widely researched area of negative emotional experiences on immune responses to vaccination. In the following sections, the influence of stress on vaccination will be reviewed in detail and critically analysed, with a specific focus on older adult populations and their responses to influenza vaccination.

#### *2.5.1.3 Stress and Vaccination*

As outlined previously in this chapter, a plethora of research has linked acute (minutes) and chronic (days to years) stress to the neuroendocrine and immune systems. There is currently no universally agreed-upon definition of stress; however, many researchers accept the working definition that stress is the psychological or physiological response to stimuli (stressors) an individual perceives as being threatening to their well-being. Stressors can be defined as events in the environment that have the capacity to result in such experiences of stress, such as caregiving and bereavement (Folkman, 2013). Researchers can measure stress in a variety of ways, but most commonly via self-report, to capture the extent to which an individual feels stressed in a given moment or period of time (perceived stress) and/or experience of stressor events over a given period. Both perceived stress and stressor measures have been widely used by researchers interested in the relationships between stress and immunity, however perceived stress is particularly valuable because it accounts for the fact that different people may find the same event (stressor) to be more or less stressful. For example, bereavement is an undoubtably stressful life event but one that can affect the stress levels of individuals differently (Phillips et al., 2006). Both definitions of stress can be measured on an acute scale (e.g., over the last week) or chronically (e.g., over the last year).

A large seminal, meta-analytic review of over 300 studies examining the relationships between stress and immunity concluded that acute and chronic stress influence the immune systems in different ways (Segerstrom & Miller, 2004). For example, one study found that acute stressors (i.e., acute exercise or mental stress) have been demonstrated to have an immune enhancing capacity by improving antibody responses to influenza vaccination (Edwards et al., 2006). Interestingly, Edwards et al. (2006) also measured IL-6 levels and found that post-acute stressor IL-6 levels was a significant predictor of A/Panama antibody

responses in women. In contrast, multiple reviews have consistently concluded that high levels of chronic psychological stress were associated with poorer antibody responses following vaccination (Burns et al., 2003; Cohen et al., 2001; Goodwin et al., 2006; Pedersen et al., 2009). This relationship between stress and the down-regulation of immune responses was consistently observed in older adult populations due to their compromised immune systems (Glaser et al., 1998; Kiecolt-Glaser et al., 1996; Moynihan et al., 2004; Phillips et al., 2006; Segerstrom et al., 2008; Vedhara et al., 1999). There are a small number of studies, highlighted in *Table 2.1* and described in more detail below, that have specifically investigated the influence of stress on influenza vaccination responses in older adult populations. The majority of these studies have focused on chronic stress, where only one study assessed acute stress (Moynihan et al., 2004). Stress characteristics for these studies can be categorised into either chronic stress of caregiving (*n*= 5), life events, such as bereavement, (*n*= 2) or acute stress measured on the day of vaccination via perceived stress (*n*= 1). Many of these studies focused on elderly informal caregivers, who are a group frequently subject to chronic stress, and compared them to non-caregiver controls.

**Table 2.1**: Characteristics of studies investigating the influence of stress on influenza vaccination responses in older adult populations.

| **Study** | ***N*** | **Average Age** | **Stress Type** | **Virus Strains** | **Immune Measures** | **Summary of Findings** |
|---|---|---|---|---|---|---|
| (Kiecolt-Glaser et al., 1996) | 32 caregivers<br>32 controls | 73.1 | Chronic, caregiving | A/H1N1: Beijing<br>A/H3N2: Texas<br>B: Panama | Antibodies (ELISA, HAI) [a] Baseline, 4-weeks post-vaccination (and 6-weeks for non-responders at 4-weeks)<br>Cytokines (IL.1 IL-2 β, IL-6)<br>T lymphocytes and monocytes | Caregivers (ELISA 38%, HAI 50% [b]) showed a poorer antibody response to influenza vaccination compared with controls (ELISA 66%, HAI 75% [b]). No differences in T lymphocyte (CD3+, CD4+ and CD8+) or monocyte percentages. Caregivers had significantly lower levels of IL-2 and IL-1β. |
| (Glaser et al., 1998) | (1)<br>32 caregivers<br>68 controls<br><br>(2)<br>32 caregivers<br>22 bereaved<br>68 controls | 72.9 | Chronic, caregiving<br><br>Life events, bereavement and chronic | A/H1N1<br>A/H3N2<br>B | Antibodies (ELISA, HAI) [a] Baseline and 4-weeks post-vaccination<br>Virus specific T-cell responses | (1) Caregivers showed a poorer antibody response, virus-specific T cell response and lower levels of IL-2 compared with controls.<br>(2) Bereavement (32% [b]) and caregiver (38% [b]) groups displayed significantly lower antibody responses to at least one of the three vaccine viral stains compared with controls (59% [b]). Bereaved former caregivers had equivalent or lower levels of T cell and IL-2 levels compared with caregivers and controls. |
| (Vedhara et al., 1999) | 50 caregivers<br>67 controls | 73 | Chronic, caregiving | A/H1N1: Johannesburg<br>A/H3N2: Nanchang<br>B: Harbing | Antibodies (ELISA) [a] Baseline, 1-week, 2-weeks and 4-weeks post-vaccination<br>Cortisol | Caregivers (16%) had a significantly lower antibody response compared with controls (36%). Stress associated with significantly increased saliva cortisol. |

| | | | | | | |
|---|---|---|---|---|---|---|
| (Moynihan et al., 2004) | 37 older adults in nursing homes | 84 | Acute, perceived stress | A/H1N1: New Caledonia<br>A/H3N2: Panama<br>B: Hong Kong | Antibodies (HAI) Baseline and 3-weeks post-vaccination | No significant correlations of post-vaccination antibody concentrations and acute stress were observed. |
| (Phillips et al., 2006) | 184 older adults | 74.6 | Chronic, life events, bereavement | A/H1N1: New Caledonia<br>A/H3N2: Panama<br>B: Shandong | Antibodies (HAI) [a] Baseline, 4-weeks and 12 months post-vaccination | Bereavement predicted poorer antibody responses for A/Panama and B/Shandong compared with controls. Marital satisfaction was associated with increased A/Panama responses. |
| (Segerstrom et al., 2008) | 14 caregivers<br>30 controls | 74.5 | Chronic, caregivers, repetitive thought | A/H1N1<br>A/H3N2<br>B | Antibodies (HAI) Baseline and 4-weeks post-vaccination<br>Cytokines (IL-6) | No association between caregiver status and antibody titres to vaccination. Negative repetitive thought predicted higher levels of depression and lower levels of vaccine-specific antibodies compared with neutral repetitive thought in caregivers. |
| (Wong et al., 2013) | 55 caregivers<br>61 controls | 72 | Chronic, caregiving | A/H1N1: New Caledonia<br>A/H3N2: Wisconsin<br>B: Malaysia | Antibodies (HAI) [a] Baseline, 6-weeks and 12-weeks post-vaccination<br>Lymphocyte subsets<br>Cytokines | No differences in antibody levels observed between caregivers and controls. Caregivers displayed elevated IL-6, with higher stress levels predicting higher IL-6 levels. Caregivers shown to have significantly lower T helper/suppressor. |

[a] 4-fold increase in antibody titres to define participants as “responders” and “non-responders”.

[b] Percentage of 4-fold increases in antibody responses, whereby participants were defined responders.

Antibody responses measures: HAI = haemagglutinin inhibition assay and ELISA = enzyme-linked immunoassay.

The majority of these studies controlled or otherwise accounted for health-related behaviours, such as alcohol consumption, sleep, exercise and body mass index, meaning there was a greater confidence that any immunological affects were attributable to stress levels. Kiecolt-Glaser et al. (1996) conducted the first ever study investigating the relationship between psychological stress and influenza vaccination responses in older adults. This study found that older adults subjected to chronic stress, in this case, caregiving for a spouse with progressive dementia, showed a poorer antibody response to influenza vaccination relative to age-matched control subjects. This was based on measuring a 4-fold increase from pre- to post-vaccination antibody responses, whereby this measure was used to determine whether an individual had a significant increase in viral-specific antibodies and classified participants as "responders" or "non-responders". This is a traditional method in the literature of measuring the change of antibody levels in vaccination studies to determine whether or not participants respond to vaccination (Glaser et al., 1998; Kiecolt-Glaser et al., 1996; Vedhara et al., 1999). In this study, researchers exclusively included participants who had received the previous year's influenza vaccination to control for recent vaccination history. This was mainly due to the fact that viral strains are often repeated in influenza vaccinations and enabled a degree of comparability of baseline antibody levels of the caregiver and non-caregiver groups. This study reported that only 38% of caregivers had a 4-fold increase in antibody titre measured by Enzyme-Linked Immunosorbent Assay (ELISA), compared with 66% of non-caregivers. This difference was magnified in older adults (>70 years old) with only 26% of caregivers responding compared to 60% in the non-caregiver group. Hemagglutinin Inhibition (HAI) assay analyses indicated similar group differences with 4-fold antibody response rates at 50% and 75% for caregivers and non-caregivers respectively (statistically significant group differences reported). The results illustrated, using two independent methods for measuring antibody responses, that caregivers showed a poorer response compared with non-caregivers and highlighted the important influence of stress on the protection of older adults against influenza. Vedhara et al. (1999) expanded on this research, by conducting a similar study in the older adult caregiving and non-caregiving populations investigating the potential mediating role of the HPA axis, as indexed by levels of cortisol in saliva, along with antibody responses to vaccination. As mentioned previously in this chapter, the HPA axis was identified as a key pathway capable of mediating the relationship between chronic stress and immune responses. In this study, increased distress was associated with significantly raised saliva cortisol concentrations, where the data suggested an inverse relationship between HPA axis activity and antibody response to

vaccination, implying the HPA axis could be a possible mediating mechanism. As with the study by Kiecolt-Glaser at al. (1998) this study found that elderly caregivers of spouses with dementia had significantly lower antibody responses to influenza vaccination, with only 16% of caregivers displaying a 4-fold increase in antibody titre from baseline for at least one of the vaccine viral strains compared to 36% in non-caregiver controls. Examining strain-specific responses in more detail, Vedhara et al. (1999) found that caregivers had significantly poorer antibody responses to the Nanchang strain (A/H3N2) compared to non-caregivers, however no group differences were observed for the Harbin (B) and Johannesburg (A/H1N1) strains. A possible explanation for this outcome could be due to previous exposure to these two stains, either via natural or vaccine exposure, where they have been noted to be present in the influenza vaccine administered in the year prior to this study. Previous exposure to Harbin (B) and Johannesburg (A/H1N1) strains may have resulted in a more robust antibody response to vaccination, therefore mitigating any group differences. These were two of the earlier studies that identified the chronic stress of caregiving to play a critical role in the immune suppression of antibody responses to vaccination in older adult populations.

Research following this also looked into the chronic stress of caregiving, but went a step further by also exploring life event stress, such as bereavement. Unconventionally, Glaser et al. (1998) conducted two studies over the course of two vaccination seasons. In the first study, Glaser et al. (1998) compared antibody levels in caregiver and non-caregiver groups pre- and post- influenza vaccination and found that caregivers had a lower proportion of pre-vaccination antibodies compared with non-caregivers, despite all included study participants receiving the influenza vaccine in the 3 years prior. Accounting for vaccination history is quite common in the literature, whereby only those who have previously received an influenza vaccine are eligible for study participation or to ensure vaccination history is recorded to measure group differences at baseline. The second study conducted by Glaser et al. only included participants who had received the previous year's influenza vaccination to ensure the groups had comparable vaccination histories. Following vaccination, the outcome of Glaser et al. (1998)'s first study found that caregivers post-vaccination showed a poorer antibody response and virus-specific T cell response compared to non-caregivers. A more rapid decline of virus-specific T cells responses over time was also observed in the caregiver group. Glaser et al. (1998)'s second study investigated former caregivers who had recently experienced bereavement in addition to current caregivers and non-caregivers as the control group. Despite comparable baseline antibody levels between the groups, the bereavement and caregiver groups displayed significantly lower antibody responses to at least one of the

three vaccine viral stains, with only 32% and 38% showing a 4-fold titre increase respectively, compared to 59% in the non-caregiver control group. Participants who demonstrated a 4-fold increase also had virus-specific T cell and IL-2 measures assessed, with bereaved former caregivers displaying equivalent or lower levels compared to both current caregivers and controls. In both of these studies, researchers were able to highlight the detrimental impact of both chronic stress and life event stress as psychological influences capable of altering cellular and humoral immune responses to influenza vaccination in older adults.

While the above caregiver studies demonstrate poorer responses among chronically stressed carers compared to age-matched controls, they do not provide much insight into the individual differences observed between caregivers. Segerstrom et al. (2008), sought to address this gap by investigating two different types of repetitive thought (defined as frequent prolonged thoughts about oneself and environment, such as worry and reflection) and how they may alter the perception of stress for caregivers and subsequently mediate immune responses to influenza vaccination. This study did not find any association between caregiver status and antibody titres to any of the influenza vaccine components. However, both negative and neutral repetitive thought were shown to interact with caregiving status to predict antibody titres, where negative repetitive thought (e.g., worry) predicted higher levels of depression and lower levels of vaccine-specific antibodies compared with neutral repetitive thought (e.g., reflection) in caregivers. The authors suggested this could be due to these negative repetitive thought caregivers amplifying stress levels, compared to neutral repetitive thought caregivers who better adjusted and processed their stress levels and, as a result, displayed increased antibody levels. Caregivers experiencing neutral repetitive thought displayed increased antibody titres, whereas little effect was seen on antibody titres for controls. In sum, caregiver antibody titres increased with increasing neutral repetitive thought, whereas control antibody titres decreased with increasing negative repetitive thought. The results of this study suggested that the influence of repetitive thought on antibody responses to vaccination were dependent on the type of thought, as well as caregiving status. Repetitive thought was capable of modifying the perception and effects of stress with negative repetitive thought being associated with poorer immune outcomes and neutral repetitive thought being associated with better immune outcomes.

In addition to the psychological stress of caregiving, stressful life events such as bereavement, an event commonly experienced by older adults, and marital status have been found to influence antibody responses to influenza vaccination (Phillips et al., 2006). Specifically, research has found that older adults who experienced bereavement in the year

prior to vaccination presented significantly poorer 4-week post-vaccination antibody levels for A/Panama (A/H3N2) and B/Shandong (B) compared to age-matched controls. An interesting observation is that across stress studies on influenza vaccination H3N2 strains seem to be more strongly influenced by chronic stress. Like Vedhara et al. (1999), this research by Phillips et al. (2006) has indicated H3N2 influenza strains to be subject to the most variation between chronically stressed and control groups. A possible reason for this could be because the H3N2 influenza strain evolves most rapidly and is considered to be critical in influencing high mortality and morbidity due to immune responses to this strain being less robust (Petrova & Russell, 2018). Antigens that elicit a less robust immune response are more likely to be subject to individual variation, and therefore allows for small to medium effect sizes to emerge (Cohen et al., 2001; Phillips et al., 2005). Studies have also found there was a trend towards lower antibody responses to H3N2 strains for participants without pre-vaccination immunity (Wong et al., 2013). Some of the literature (Glaser et al., 1998; Kiecolt-Glaser et al., 1996) reported participants as responders if they demonstrated a 4-fold increase from pre- to post-vaccination antibody titres for either one of the three individual vaccine components or for all three components. However, this means that some researchers would report overall differences, but would not report differences for specific strains making it impossible to gain insight into whether similar strains were shown to be more sensitive to the influence of chronic stress.

Gender throughout the literature in this section focusing on older adults did not stand out as being a confounding factor when investigating antibody responses to vaccination, however there has been some evidence to suggest healthy young adult women have a poorer response to A/Panama (A/H3N2) strains than men (Phillips et al., 2005). Phillips et al. (2005) also found that being married and having high marital/ cohabiting satisfaction were associated with higher 4-week antibody responses to one of the influenza strains (A/Panama (A/H3N2)) ($p$=0.03). This suggested that responses to this specific influenza strain was not only sensitive to the influence of the negative effects of stress, but could also be positively influenced by factors such as being married, high marital/cohabiting satisfaction and being a male. This study showed the protective effects of being married compared with single/separated/divorced/widowed individuals, as well as the detrimental immunological effects due to the stress of bereavement.

Several studies reviewed in this section have investigated a variety of cytokine measures, in addition to antibody measures, to assess immunological differences between caregiver and non-caregiver groups. For example, Kiecolt-Glaser et al. (1996) took into account cytokine

measures (IL-1β, IL-2 and IL-6) as they are capable of serving as T- and B-lymphocyte amplifiers. Percentages of T lymphocytes and monocytes were also considered to ensure any difference in cytokine responses were due to cellular responses to stimulation (vaccination) instead of group differences. No differences in T lymphocyte (CD3+, CD4+ and CD8+) or monocyte percentages were found, however caregivers demonstrated significantly lower levels of virus-specific-induced cytokines (IL-2 and IL-1β), further highlighting the downregulated immune response to vaccination when subject to chronic stress. Consistent with these findings, Glaser et al. (1998) also found caregivers displayed lower levels of virus-induced IL-2. As mentioned previously, IL-6 has been identified as a hallmark for depression, and research by Segerstrom et al. (2008) has found this to be the case in the context of stress. In alignment with other research in this area (Ershler & Keller, 2000; Mastorakos & Ilias, 2006; Papanicolaou et al., 1998), Segerstrom et al. (2008) found caregivers at baseline illustrated higher levels of IL-6, as well as increased IL-6 levels post-vaccination, compared with controls. Controls on the other hand demonstrated an IL-6 decrease from pre-vaccination to 4-weeks post-vaccination. Increasing levels of negative repetitive thought was associated with increased IL-6 for caregivers and decreased IL-6 for controls. This evidence identified chronic stress experienced by elderly caregivers to be associated with increased vulnerability to infectious diseases via elevated IL-6.

Throughout this chapter, and in the literature outlined above, there has been strong evidence to outline the impact of depression, negative affect and chronic stress on influenza vaccination responses. In addition to this, IL-6 has frequently been assessed and has been found to be associated with these psychological factors. This mechanism of IL-6 contributing towards the downregulation of immune responses is unsurprising given the evidence of its mechanism as a pro-inflammatory mediator capable of modulating immune responses. Earlier in this chapter, the relationship between stress, the HPA axis and immune system was outlined. Specifically, the activation of the HPA axis, due to stress, leading to the secretion of glucocorticoids that have the ability to bind to the surface of immune cells, such as T cells, to upregulate the production of pro-inflammatory cytokines (IL-6). IL-6 is a well characterised pro-inflammatory cytokine that is capable of binding to cytokine receptors on the surface of immune cells to initiate the Janus kinase-signal transducer and activator of transcription (JAK-STAT) signalling pathway. The chronic activation of this pathway, via pro-inflammatory cytokine (IL-6) receptor binding, has been identified to contribute towards the pathogenesis of chronic inflammatory (e.g., cancer) and autoimmunity diseases (e.g., atopic dermatitis, rheumatoid arthritis) (Tanaka et al., 2014; Tsukamoto et al., 2018). The downstream effects

of JAK-STAT pathway activation can lead to the transcription of pro-inflammatory mediator genes (IL-4, IL-13, IL-22) that are key contributors towards the progression of disease. This IL-6 JAK-STAT signalling pathway is so influential on disease and health related outcomes that it has been a target for many therapies, either via pro-inflammatory cytokine receptor inhibition or via intracellular associated JAK inhibition (Aittomäki & Pesu, 2014). This highlights one of the important cellular immune mechanisms influenced by stress that can contribute towards the increased immune vulnerability of chronically stressed individuals, particularly stressed elderly caregivers. This indicated that the highlighted immune response differences, particularly IL-6, observed between caregivers and non-caregivers are important to take into consideration when investigating the influence of stress on the immune activity of older adults.

The literature outlined above provides evidence of the impact of chronic stress, whether that be caregiving or bereavement, on influenza vaccination responses in older adults. However, only one study to date has examined the impact of acute stress on influenza responses in older adults. This study, in contrast to those outlined above, focused on the measurement of perceived stress (degree to which current life events are stressful) and depressive symptoms over the last week in older adults in a nursing home, rather than specific stressors, such as bereavement and caregiving (Moynihan et al., 2004). All participants received the previous year's influenza vaccination and therefore had comparable baseline levels of each vaccine virus strain present. A 4-fold increase in antibody concentration was observed for 45.6% of the New Caledonia (A/H1N1) strain, 59.6% of the Hong Kong (B) strain, 62.2% of the Panama (A/H3N2) strain and 55% for all three strains. These values were notably higher than the percentages observed by the other studies and was also slightly higher than the typical vaccination response range for older adults (18-50%) (Govaert, 1994; P. A. Gross et al., 1995). A significant limitation of this study was that it did not have a control group. Instead, they compared perceived stress scores and responses to each vaccine virus strain to identify correlations between the two. Perceived stress was negatively correlated with pre-vaccination antibody concentrations of the New Caledonia and Hong Kong strains, but not the Panama strain, suggesting perceived stress could be correlated with the rate of decline of pre-existing antibodies. However, this study showed no effect of stress on post-vaccination antibody levels. This is not too surprising given the evidence of acute stress acting in an immunoenhancing manner by increasing dendric cell, neutrophil, macrophage and lymphocyte trafficking, maturation and function and has been shown to augment innate and adaptive immune responses (Edwards et al., 2006). Evidence has indicated that acute stress

experienced prior to antigen exposure (keyhole limpet hemocyanin) in animals can increase memory T cell production to result in a significant and long-lasting increase in immunity (F. S. Dhabhar & Viswanathan, 2005).

In sum, Moynihan et al. (2004) was unable to demonstrate that acute stress in older adults was capable of influencing influenza vaccination responses, however several methodological issues suggest these findings should be treated with caution. This study assessed antibody responses at 3-weeks post-vaccination, contrasting with other literature that has measured short-term antibody responses at 4-weeks post-vaccination (Glaser et al., 1998; Kiecolt-Glaser et al., 1996; Phillips et al., 2006; Segerstrom et al., 2008; Vedhara et al., 1999). Prior research indicated that peak antibody levels are reached at around 28-days, meaning 4-weeks post-vaccination is arguably a more justifiable endpoint (P. A. Gross et al., 1997). Additionally, this study did not have a control group making it difficult to draw accurate conclusions about whether antibody responses to influenza vaccination were at all modulated by acute stress.

Unfortunately, limited literature makes it hard to draw conclusions on the relationship between acute stress and immune responses to influenza vaccination in older adults. One of the reasons for this is, perhaps, due to the practical and ethical issues presented when conducting research with older adult populations, such as acute stress-inducing activities. There are, however, studies conducted on other populations that suggest a relationship between acute stress and vaccination responses. For example, acute stress exposure prior to influenza vaccination enhanced antibody responses in healthy young adults (Edwards et al., 2006). Stress-inducing tasks included an exercise task involving cycle ergometer activities lasting 16-minutes in total and a mental arithmetic task lasting 45-minutes that subjected participants to loud bursts of noise in response to incorrect answers. This study provided some of the first evidence of acute stress-induced immunoenhancement in humans, whereby women in particular, in both the exercise and mental stress conditions, were shown to produce higher levels of A-Panama (A/H3N2) antibodies while men responded similarly in all conditions. The mental arithmetic tasks were associated with elevated IL-6 and cortisol levels, which were also shown to be associated with exercise stress duration and intensity. Despite the elevation of cortisol during acute stress-inducing tasks, there was no significant association with post-vaccination antibody responses. This suggested the importance of cortisol and vaccination responses is critical for chronic stress, may not be as important for acute stress-induced immune modulation.

As noted previously, evidence has shown that acute stress is related to increased levels of glucocorticoids and the pro-inflammatory cytokine IL-6 thus resulting in the enhancement of antibody responses to vaccination. Lee et al. (1999) for example identified co-administration of IL-6 gene with DNA-based viral influenza vaccination completely protected mice from future virus challenge exposure, therefore suggesting IL-6 was capable of inducing long-term protective immunity. Additionally, another study found that healthy adults vaccinated with a live virus strain and classified as responders had higher levels of IL-6 pre-vaccination compared with non-responders (Edwards et al., 2006). Edwards et al. (2006) also identified a significant association between IL-6 and A/Panama (A/H3N2) levels 4-weeks post influenza vaccination in young women, further highlighting the protective capacity of acute stress and IL-6 levels prior to vaccination. The vaccination adjuvant properties of IL-6 could be explained by its role in the inhibition and polarisation of T helper cells (Th1) and differentiation and stimulation of type 2 T helper cells (Th2) (Diehl & Rincón, 2002). Th2 cell stimulation is an important part of the innate immune response due to activation-induced secretion of IL-4, IL-5, IL-10 and IL-13, which mediate B cell activation and antibody production (O'Garra, 1998). Whilst Edwards et al. (2006) did not identify IL-6 as a key mediator of antibody responses and suggested this cytokine more likely served as an indicator of the extent of the inflammatory response to acute stress tasks, it did suggest that IL-6 could play a role in humoral immune responses. However, it is important to highlight the drastically different effects of IL-6 on the immune system in an acute stress and chronic stress context. Whilst in the context of acute stress IL-6 can have immunoenhancing effects capable of upregulating antibody production, its chronic production via chronic stress (as described earlier in this chapter) can be extremely detrimental and has been identified to contribute towards disease pathogenesis (Tanaka et al., 2014). While cytokines have been studied in the context of acute stress and immune modulation, there is currently a lack of research examining whether they can act as vaccine adjuvants.

In sum, acute stress was shown to have a very different effect on the immune system in the context of vaccination responses compared to chronic stress, whereby acute stress could enhance immune responses to vaccination and chronic stress played a key role in immune suppression. However, comparatively, there is much less literature investigating acute stress compared with chronic stress in the context of vaccination so there is less confidence from the literature about its ability to affect the immune system. The literature reviewed in this section highlighted the importance of investigating stress and influenza vaccination

responses in older adults, however the research did present some limitations that are important to consider and are outlined below.

#### 2.5.1.3.1 Limitations of Literature on Stress and Vaccination

A limitation of some of the literature around stress and vaccination responses is that some studies did not fully account for prior antigen exposure, whether via infection or vaccination, which meant that baseline measures could be skewed. The nature of influenza infection means that even the minority of individuals who were not vaccinated (typically over 70% influenza vaccination uptake is reported each year but this is recently been shown to increase to 91.2% as a result of the COVID-19 pandemic (Bachtiger et al., 2021)) were likely to have encountered natural exposure and would display influenza-specific antibodies prior to vaccination. For example, participants with previous exposure will display high baseline antibody levels but may not, as a result, achieve a 4-fold antibody increase post-vaccination. As a result, these participants would be classified non-responders despite having high antibody levels, which would confer protection. Failure to account for previous exposure can mask the magnitude of the impact of stress on immune and vaccination responses due to the inability to accurately record differences between pre- to post-antibody responses to vaccination. This is particularly important for research into older adults who are very likely to have had previous exposure and are therefore mounting a secondary immune response to vaccination. Although most influenza and stress studies do not provide enough evidence to determine whether they assessed primary or secondary responses, fortunately, the nature of the older adult age group mean researchers can assume that secondary immune responses are being assessed as a result of previous influenza exposure either via natural viral exposure or via previously administered vaccinations. The consequence, however, is that very little is known about primary responses in the influenza vaccination literature.

The majority of studies focused on measuring 4-week post-vaccination antibody responses determine responders and non-responders, due to this timepoint being recognised to assess peak antibody levels, one study went on to measure 6-week post-vaccination antibody responses and found there were a higher proportion of responders compared to 4-weeks (Glaser et al., 1998). This is important to take into consideration because it took longer for these participants to respond to vaccination than the conventional 4-week timeframe routinely cited in the literature. It demonstrated the value of extending the antibody measurement timeframe to maximise the data captured by ensuring participants were not incorrectly classified as responders and non-responders. This is a general limitation of the

majority of the literature outlined in this section, whereby the majority of antibody outcomes were assessed at 4-weeks post-vaccination.

Another limitation was that many of the studies described above did not assess both cellular and humoral-mediated immune responses to influenza vaccination. Whilst humoral-mediated immunity, specifically antibody responses, are conventionally measured for assessing responses to vaccination, the cellular immunity of T cell and cytokine environments are also important to assess. Cellular immunity in the context of vaccination is important to consider when researching older adult populations due to the impact of ageing on cell-mediated immunity, which is significantly more adverse than humoral mediated immunity (McElhaney et al., 2006; Thorpe et al., 2006). Evidence suggests that the protective capacity of vaccination is dependent on their ability to induce both cellular and humoral immunity in older adults (Govaert, 1994). For example, caregivers with higher stress levels showed higher levels of inflammatory cytokines (IL-6, IL-10 and IL-8), which is an indicator of depression and poor health in older adults (Kiecolt-Glaser et al., 2007). Usually IL-6 levels are tightly regulated and expressed at low levels until influenced by infection and/or stress. High levels of IL-6 are particularly relevant for the health of older adults, with elevated IL-6 levels being shown to increase morbidity for several diseases associated with ageing (e.g., cardiovascular disease, osteoporosis, Alzheimer's disease) and has also been shown to contribute towards old age frailty by decreasing lean body mass and low-grade anaemia (Ershler & Keller, 2000; Mastorakos & Ilias, 2006; Papanicolaou et al., 1998). Post-vaccination, elderly caregivers were observed to have elevated IL-6 levels from pre-vaccination and may contribute towards more humoral-mediated immune responses (Segerstrom et al., 2008).

It has also been argued that antibody responses may not be the best correlate of protection for influenza in older adults and that T cell mediated responses could be better (McElhaney et al., 2006). For example, McElhaney et al. (2006) made some valuable arguments about the importance of taking into consideration T cell responses to vaccination. This prospective study assessed antibody titres and T cell responses in vaccinated adults followed throughout the influenza season to detect influenza illness. In addition to T cell responses, Th1 and Th2 cytokine ratios of IFNγ:IL-10 were evaluated to gain insight into the anti- and pro-inflammatory cytokine profiles of adults with laboratory diagnosed influenza. These findings indicated that laboratory diagnosed influenza patients had a lower ratio due to the under production of IFNγ and overproduction of IL-10, therefore reflecting a shift towards a Th2 response and was identified as a potential marker for determining risk for influenza illness. This evidence highlighted the importance for future influenza vaccination research in older

adult populations to consider cellular immunity, such as T cell responses and cytokines measures, in addition to antibody responses to gain a better overall understanding of vaccination responses.

One study, however, did conduct a study to assess both humoral-mediated and cellular-mediated immune responses to influenza vaccination. Wong et al. (2013) found that elderly caregivers, who were reported to have higher stress levels than matched controls, had decreased lymphocyte (cell-mediated) and cytokine immune responses to influenza vaccination. Crucially however, they observed no differences in humoral-mediated (antibody) immune responses to vaccination between caregivers and controls. This was an important comparison of immune findings because it suggested that research in this field should look beyond humoral-immunity to also consider cellular immunity when investigating immune functioning. In addition, Wong et al. (2013) also measured vaccine-specific antibody titres, lymphocyte subsets (absolute counts of total T lymphocytes, T helper, T suppressor, cytotoxic T, natural killer cells, and B lymphocytes), as well as cytokines (IL-1β, IL-6, IL-8 and TNFα) measured via lymphocyte stimulation to test for immunocompetence. This was particularly important to assess given the evidence of increased vulnerability to influenza infection being associated with cytokine responses in older adults (Ershler & Keller, 2000; Mastorakos & Ilias, 2006; Papanicolaou et al., 1998). Wong et al. (2013) found that caregiver baseline levels of pro-inflammatory cytokines IL-10, IL-6, IL-1β and IL-8 were significantly higher when compared with controls, and IL-6 in particular was higher for caregivers with higher stress levels. Caregivers were also shown to have significantly lower T helper/suppressor ratio from baseline to 12-weeks post vaccination compared with controls, which suggested a lower cell-mediated immune responses upon activation. In contrast to the previous studies, researchers were unable to demonstrate proportion differences between responders and non-responders for caregivers and noncaregivers. This study was informative because it suggested that high levels of chronic stress were associated with high IL-6 levels, which subsequently had a negative effect on antibody responses to influenza vaccination, making older adults more vulnerable to infection. However, this same cytokine expressed immediately prior to influenza vaccination, as a result of acute stress, has been indicated to improve antibody responses and induce protection against future virus exposure (Krakauer, 1995; S. W. Lee et al., 1999). This suggests that IL-6 may work in a similar capacity to cortisol, whereby high levels for a prolonged period of time are detrimental to the immune system, but acute bursts could be extremely beneficial and contribute to immunoenhancement.

Prolonged, repetitive exposure to stress and subsequently IL-6 levels could also be a biological mechanism to explain allostatic load (Carpenter et al., 2010).

Other factors that would be worth taking into consideration for studies investigating influenza vaccination responses include time of day of vaccine administration. Some literature has suggested that vaccination responses could be modified depending on the time of day of antigen administration. This could be due to differences in peak pro-inflammatory cytokine release and/or fluctuations in cortisol levels that are reported to be at their lowest during the night and in the early hours of the morning (Long et al., 2016; Petrovsky & Harrison, 1998). Long et al. (2016) demonstrated that the simple manipulation in the timing of vaccination administration to favour morning, as opposed to afternoon, proved to be beneficial for influenza vaccination antibody responses in older adults. There has also been research that showed immune system stimulated at different times of the day resulted in peak dendritic cell migration in the morning (Holtkamp et al., 2021). Dendritic cell migration from the skin and other organs through the lymphatic vessels to the lymph nodes is important for antigen presentation as this is the first step in mounting a specific immune response. This suggested that the co-administration of immune-regulatory hormones, such as catecholamines (e.g., epinephrine and norepinephrine), as well as time of day or vaccine administration could be feasible therapeutic methods of improving immune responses to vaccination.

The evidence surrounding the influence of chronic stress, such as caregiving and bereavement, on influenza vaccination responses in older adult populations has consistently found chronic stress to result in immunological impairment. This suggested that chronic stress is not only associated with influenza vaccination responses, but also significantly influenced the protective capacity of vaccination, making older adults more vulnerable to infection. However, it is crucial for future research into chronic stress and immune responses to vaccination to consider the importance of cellular-mediated immune response in addition to humoral-immunity. Acute stress, however, has been indicated to have an immunoenhancing influence highlighting the differences of immune system enhancement or suppression being dependent on stress duration. In the following section of this chapter, positive emotional experience and its impact on immune responses will be discussed.

### *2.5.2 Influence of Positive Emotional Experience on the Immune System*

In contrast to negative emotional experience, such as stress, the evidence around positive emotional experience, such as positive affect, and influenza vaccination responses is less

abundant. It is only in recent years that the attention of research has examined positive affect and physical health outcomes. In the previous sections, the literature outlined has primarily focused on older adult populations and the influenza vaccination because they form the foundation topics of this thesis. However, in this section, due to limited research investigating positive emotional experience in the context of older adults and influenza, a broader set of literature focusing on other populations, vaccination types and immune parameters will be reviewed in the context of positive affect. The majority of this literature outlines immune parameters and observational evidence in the context of positive affect meaning much of this evidence is correlational as opposed to causal. Positive affect is defined as emotional experiences of enthusiasm, alertness and pleasure. Other positive psychological constructs used in immunity and health studies that have similarities to positive affect include self-esteem, optimism, as well as more complex measures like quality of life and well-being that can be categorised as being positive emotional experiences (Ryff & Singer, 2003; Salovey et al., 2000; Yu & Clark, 2015). Multiple reviews have examined the psychobiological relationship between positive affect and the immune system (Barak, 2006; Dockray & Steptoe, 2010; Marsland et al., 2007; Steptoe et al., 2009). Characteristically high positive affect levels have been linked to lower hospital readmission, reduced risk of stroke and has been shown to predict lower mortality in older adults (Middleton & Byrd, 1996; Ostir et al., 2001). A review of the literature outlining the immune and health benefits of positive affect will be discussed in this section.

The beneficial effects of positive affect have been shown to be related to improved health related outcomes, such as cardiovascular and immune functioning, whereby duration of positive affect is important to consider. In the same way that stress can be acute and chronic, positive affect durations can also vary with trait affect and state affect indicating long-term and short-term emotional experiences, respectively. In addition to trait positive affect, state positive affect has also been linked to skin responses to allergens. For example, Laidlaw et al. (1996) found that feelings of pleasantness and relaxation induced by hypnosis resulted in a significantly reduced allergic response to histamine. Feelings of irritability, tension and high blood pressure readings were also associated with a smaller reduction in allergy responses, further highlighting the role of positive affect in the regulation of skin responses to allergies. There has also been research to induce positive affect, whereby laugher after watching a comedy film was linked to decreased pollen-specific IgE and IgG antibody levels, as well as reduced TNFα (Kimata, 2004). This suggested that inducing positive affect may have some implications for the treatment of allergic disease.

Whilst positive and negative affect are considered by some to be independent constructs, some evidence has suggested an association of affect with stress (Herbert & Cohen, 1993). Herbert and Cohen (1993) suggested that individuals with high trait negative affect scores were more prone to psychological distress, both of which have been associated with compromised immune function. Research indicated that in the same way trait negative affect and stress are related, trait positive affect may be related to stress by acting as a buffer for the effects of acute stress (Robles et al., 2009). Robles et al. (2009) investigated the role of self-report trait positive affect on skin barrier recovery after skin disruption (a "tape-stripping" procedure that disrupts normal skin barrier function). Trait positive affect was assessed for participants prior to them undergoing an acute psychological stressor (a 5-min speech and 5-min mental arithmetic task), whereby individuals with greater trait positive affect showed faster skin barrier recovery. However, positive affect was not significantly related to skin barrier recovery for participants who did not undergo a psychological stressor, therefore supporting the notion of trait positive affect acting as an acute stress buffer. This study also took into account trait negative affect measures to ensure the effects of trait positive affect on wound healing were not driven by the effects of negative affect. The effects of trait positive affect on skin barrier recovery were found to be independent of the effects of trait negative affect, which was important to note due to the evidence supporting the relationship between negative affect and poor health outcomes (Kiecolt-Glaser et al., 2002). The findings of this study suggested that trait positive affect was capable of buffering the effects of acute stress on wound healing. A possible explanation could be alterations of neuropeptide and neurotransmitter released in the skin that have previously been linked to the neuroimmunology response of stress (Arck et al., 2006). Ultimately, the biological mechanism of trait positive affect acting as a buffer to aid skin barrier recovery is unknown and requires more exploration. The investigation of these possible biological mechanisms could be extremely interesting and may offer some insight into potential avenues for buffer the effects of stress on wound healing and other types of immune dysregulation.

#### *2.5.2.1 Well-being and Immunity*

Interest in examining the correlation between positive affect and immunity stems from the plethora of positive well-being and health research, whereby increasing amounts of research have shown that psychological well-being is related to better physical health (Howell et al., 2007). The construct of well-being is very complex and can incorporate a variety of factors including purpose in life, personal growth and purposeful engagement in life (Ryff et al., 2004). A prospective epidemiology cohort study demonstrated that, over a 15-year

timeframe, participants with higher scores of positive well-being traits were at a notably reduced risk for coronary heart disease (Kubzansky & Thurston, 2007). Additionally, a review of the evidence suggested that positive psychological well-being had a favourable effect on mortality rates of both healthy and diseased populations (Chida & Steptoe, 2008). In addition to the influence of well-being on physical health, converging evidence suggested that positive affect was capable of neuroendocrine, autonomic and immune system activation and, in contrast to stress, was consistently shown to be associated with lower levels of cortisol (Pressman & Cohen, 2005; Ryff et al., 2004). Ryff et al. (2004) investigated whether the construct of well-being was capable of protective effects on the neuroendocrine, immune and overall health of older adults. Neuroendocrine function was assessed via daily saliva cortisol samples, overnight urinary epinephrine and norepinephrine and dehydroepiandrosterone-sulphate (DHEA-S) (an important compound for making testosterone in males and oestrogen in females) from blood samples. These markers were shown to be much lower in older women who reported higher levels of purpose in life and personal growth compared with those who displayed lower levels of general well-being. Interestingly, those who rated higher on the well-being spectrum started the day with lower cortisol levels that remained lower throughout the day. Researchers have found that DHEA-S and cortisol have opposing effects on the immune system, where they are immune enhancing and suppressive, respectively (Butcher et al., 2005; Murialdo et al., 2001). Unfortunately, levels of DHEA-S decrease with ageing, another example of immunosenescence, and is another mechanism for infection susceptibility for older adults. For example, Butcher et al. (2005) examined adrenocortical hormone levels in older adults with hip fractures and age-match controls. Injury infection was monitored with findings showing there were increased cortisol: DHEA-S ratios in older adults who succumbed to infection compared with those who did not. The immune enhancing effects of DHEA-S have also been outlined by Solerte et al. (1999) who found that DHEA-S can positively modulate the immune function of NK cells, with NK cell cytotoxicity being greater in healthy young subjects compared with healthy old subjects. Although the age-dependent decline of DHEA-S could leave older adults more susceptible to infection, DHEA-S exposure, perhaps via positive affect induction, might contribute towards maintaining immune cytotoxicity via NK cell activity to facilitate with infection protection (Solerte et al., 1999). This evidence for the protective effects of well-being on the neuroendocrine and immunological outcomes in older adults is modest and calls for the need for further investigation.

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#### *2.5.2.2 Positive Affect and Immunity*

Positive affect has been associated with an array of immune measures indicating immune competence. An example of this includes the increase of secretory IgA responses to antigen challenge (Stone et al., 1994). Secretory immunity is one of the most widely reported immunological measures in the context of positive affect research (Evans et al., 1993; Stone et al., 1987, 1994). One study examined the link between daily stressful events and secretory IgA antibodies in healthy adults who completed daily questionnaires and daily saliva samples for up to 12-weeks. Stone et al. (1994) demonstrated that both desirable (e.g., personal leisure events) and undesirable (e.g., work events) daily events were linked to secretory IgA antibody responses, where evidence suggested that desirable events had the potential to be stronger predictors. This was consistent with the literature that highlighted that increased positive affect after mood induction demonstrated raised secretory IgA levels (Hucklebridge et al., 2000; Njus et al., 1996; Stone et al., 1996). For example, Hucklebridge et al. (2000) subjected 19 students to mood manipulation via recall and music and assessed saliva IgA concentration and secretion rate. Both methods of mood induction were associated with increases in salivary IgA. Njus et al. (1996) also assessed the impact of positive mood induction in a cohort of 50 students and found that watching humorous videos resulted in higher secretory IgA concentrations. However, these outcomes should be interpreted with appropriate caution because the majority of these studies had a small number of participants and in some instances did not test for a significant effect of mood induction manipulation due to the absence of a control group. These findings were in contrast to Evan et al. (1993) who found no association between positive affect and secretory IgA and even suggested that negative affect is associated with higher secretion rates of secretory IgA. Two studies that found an association with positive affect and secretory IgA, and included a control condition within their study design, have also reported increased secretory IgA to be associated with negative affect (Hucklebridge et al., 2000; Njus et al., 1996). All of the above studies were suggestive of an association of positive affect and secretory antibodies, but requires further investigation.

Positive affect has also been found to impact on immune function in ways that are highly clinically relevant. For example, greater levels of positive emotional style (a measure of dispositional/trait positive affect) was shown to predict a reduced risk of illness for individuals experimentally infected with rhinovirus (Doyle et al., 2006). In this study, healthy adults were exposed to one of two strains of rhinovirus, followed by 5-days in quarantine to assess IL-1, IL-6 and IL-8 levels, whereby higher IL-6 was identified to be associated with

greater illness symptoms. Cytokines in particular are important to measure in the context of respiratory illness due to their pro-inflammatory and anti-inflammatory effects that have been identified to can act as biomarkers for infection and illness. For example, nasal IL-6, IL-8, TNFα, and INFα levels were increased in individuals displaying symptoms of illness after influenza vaccination (Hayden et al., 1998), and increased IL-1β and IL-6 were noted in symptomatic patients experimentally infected with rhinovirus-39 (Gentile et al., 2003). As discussed in the previous section (see *Section 2.5.1.3*), IL-6 is a key modulatory cytokine upregulated in the context of chronic stress and has been shown to downregulate innate and adaptive immune responses and increase an individual's susceptibility to illness (Cohen et al., 2003a). In a study by Doyle et al. (2006), higher positive emotional style was associated with lower IL-6 levels and fewer illness symptoms. Overall, positive emotional style was strongly associated with nasal and throat symptoms, with higher positive emotional style resulting in decreased symptoms of disease. This study identified the effects of positive emotional style to have the opposite effect on the immune system, in terms of IL-6 regulation, compared to chronic stress.

Another study quarantined healthy, adult volunteers to monitor the development of biologically verified upper respiratory infections after inoculation with a rhinovirus (Cohen et al., 2006). Positive emotional style was assessed in the 3-weeks prior via daily measures and found that participants with high positive emotional style were up to three times less likely to develop symptoms of infection. Interestingly, and important to note, the associations outlined above between positive affect and developing respiratory illness were shown to be independent of virus type, age, sex, education, race, body mass, season and negative affect (or negative emotional style). They were also independent of optimism, extraversion, mastery, self-esteem, purpose, and self-reported health meaning that these studies have indicated that positive emotional style played a very important role in infection susceptibility and health.

Cytokines have been identified as an important component of the immune system by playing a critical role in the activation of cellular immune responses. IL-6 was shown repeatedly to play a mediating role in the relationship between negative affect and vaccine responses. Evidence suggests it was important in the relationship with positive mood too. The inverse relationship between positive affect and IL-6, independent of covariates such as age, ethnicity, BMI, smoking and negative affect, has been outlined in a variety of studies (Friedman et al., 2007; Posner et al., 2005; Steptoe et al., 2008). For example, Friedman et al. (2007) tested the hypothesis that psychological well-being could predict lower levels of

pro-inflammatory factors and found that higher scores of positive relationships and purpose in life indicated lower IL-6 levels. What was most interesting about this research was the observed differences between males and females, whereby the association between positive affect and IL-6 was more evident in women. In addition to females being more sensitive to a decrease in IL-6 levels in response to positive affect, they were also observed to have larger IL-6 responses to stress challenge (Steptoe et al., 2002). These observed gender differences are hypothesised to be due to differences in emotional processing between men and women or to differences in bioactivation associated with emotions (Stroud et al., 2002; S. E. Taylor et al., 2000). Irrespective of gender, positive affect is seen to have the opposed effects to stress in terms of IL-6 immune responses, which raises the question about whether the induction of positive affect could be a potential option for reducing elevated IL-6 levels in chronically stressed individuals as a method of combating its immunosuppressive effects.

#### *2.5.2.3 Positive Affect and Vaccination*

Very little research has focused on the positive affect of older adults in the context of immune responses, but there is some evidence to suggest that the connection between positive affect and improved immune health can also be extended to this population. One study in particular has provided some insight into the relationships between mood, optimism and cytokine responses to influenza vaccination in healthy older adults (Costanzo et al., 2004). This study showed that greater mood disturbance was associated with lower cytokine responses to vaccination, whereas optimism and vigour were associated with greater cytokine responses. Specifically, T helper 1 cytokine responses (IFN-γ and IL-2) to vaccination were more robust in individuals with greater optimism, whereas T helper 2 cytokine responses were not associated with mood or optimism. An improvement in T helper 1 cytokine responses was particularly important due to the role of T helper 1 type responses in clearing viruses and preventing infection (Beňová et al., 2020). Interestingly, Costanzo et al. (2004) suggested that positive mood and optimism had the potential to act as a buffer for T lymphocyte functionality decreases that occur as a result of immunosenescence by modulating HPA axis activation. This was consistent with the research conducted on younger adults that observed an association between optimism and increased numbers of helper T lymphocytes (Segerstrom et al., 1998). In sum, this evidence suggested that optimism may serve as a buffer by protecting older adults against the effects of immune-ageing.

To date there has only been one study conducted to investigate the influence of positive affect on influenza vaccination responses of older adults (Ayling et al., 2018). In this prospective observational study, Ayling et al. (2018) investigated a range of behavioural

(physical activity, nutrition, sleep) and psychological (stress, positive affect, negative affect) influences and found that greater positive affect, whether measured repeatedly over a 6-week period around vaccination, or on the day of vaccination, significantly predicted greater A/H1N1 antibody responses to influenza vaccination but not for A/H3N2 and B strains. No other behavioural or psychological factors measured significantly predicted vaccination outcomes. Interestingly, it was the effects of positive affect on the day of vaccination that had a greater influence on immune responses to vaccination. However, positive affect over a longer period of time also influenced positive affect on the day of vaccination and suggested the two were not completely independent. This study was of particular importance because it not only provided evidence for the effects of positive affect as an immune modulator, but also suggested that positive affect had the potential to improve antibody responses to vaccination. It would be interesting to find out whether psychological interventions have the potential to improve immune responses to vaccination in older adults. Within the next section, an array of psychological interventions will be explored in terms of their impact on immune and vaccination responses.

## 2.6 Role of Psychological Interventions as Immune Modulators

There is an array of evidence outlined within this thesis that has suggested that stress and affect are capable of modulating immune responses, whereby the potential of harnessing these could have beneficial effects on the upregulation of immune responses for vulnerable populations, such as older adults. Older adult populations are subject to the consequences of immune-ageing, which means they conventionally respond poorly to vaccination and are therefore more susceptible to infection. Whilst pharmaceutical avenues have been explored to improve vaccine efficacy for this age group, improving efficacy has been limited and has been more frequently observed in younger adults (McKee et al., 2007). Increasing evidence of positive affect induction via the use of psychological interventions to improve immune responses has shown some promise. The types of psychological interventions that can be implemented to influence affect include a wide range of approaches including films (J. Gross & Levenson, 1995), music (Juslin & Västfjäll, 2008; Kreutz et al., 2008), images (Bradley & Lang, 2017), as well as reading and writing interventions (Hernandez et al., 2003).

### *2.6.1 Stress- Induction Interventions*

Psychological interventions can be utilised to either induce-emotion or aid with the alleviation of an emotional experience, such as stress (J. Gross & Levenson, 1995; Kreutz et al., 2008; Labott et al., 1990). They have proven to be effective for treating stress and can positively influence the course of chronic disease (Petrie et al., 1995). For example, an

intervention was developed to reduce chronic stress immediately prior to hepatitis B vaccination and found this intervention group displayed a greater increase in vaccine-specific antibody concentration (Petrie et al., 1995). This intervention aimed to decrease the stress levels of medical students who were tasked with writing about personally traumatic events during four consecutive daily sessions. The writing intervention involved participants expressing their emotions by writing about stressful topics, functioning as an emotional disclosure intervention. Psychosocial interventions have also been developed to aid with the management of stress and negative affect, such as depression. Most notable is cognitive-behavioural stress management, which was shown to improve the quality of life of individuals with chronic disease by reducing sympathetic nervous system activation and decreasing cortisol release from the adrenal cortex (Schneiderman et al., 2005). Stress management interventions have been demonstrated to be clinically relevant by positively impacting disease morbidity or mortality (Schneiderman et al., 2001). Mood-induction interventions present some ethical implications that were important to consider when investigating negative emotions, such as stress and fear. In older adult populations in particular, developing an intervention to induce stress could potentially be problematic to the already frail health of this age group.

### *2.6.2 Affect Interventions*

The nature of positive affect-inducing interventions does not present the same ethical concerns observed for stress-induction interventions. The approach of utilising interventions to induce positive mood or reduce negative mood have the potential to be incorporated into clinical settings and could serve as a cost-effective method of improving immune responses to vaccination. The aim of this section is to review the different types of psychological interventions capable of altering immune parameters, with a focus on vaccination where possible.

The duration and frequency of psychological interventions can vary drastically, whereby some are short, single-session interventions and others are repetitive and can last for several hours, days or weeks. Affect interventions can be broadly categorised as brief or non-brief. What constitutes as a brief intervention in terms of duration is a little arbitrary, which is why specifying single-session interventions as brief bypasses the issue of trying to define an exact duration end-point. By that logic, non-brief interventions are any that require more than a single-session. Within the context of busy GP surgeries, there is no doubt that brief interventions appeal as the more suitable choice from a feasibility of implementation perspective. This is because busy GP surgeries in the winter, and particularly around

influenza vaccine administration timeframes, have limited staff and facilities that can make it extremely challenging for health care professionals to manage. However, an outline of the important literature investigating the influence of non-brief interventions on immune responses, with a specific focus on vaccination responses, will be outlined below. This will be followed by the brief intervention literature to compare and contrast. The aim of this section is to access and provide insight into the possible intervention types that could be most suitable for implementation into standard vaccination clinics and to determine which are capable of acting meaningfully on immune responses to vaccination.

#### *2.6.2.1 Non-Brief Interventions and Immunity*

A systematic review has discussed in detail the potential of psychological interventions acting as vaccine adjuvants on a range of age populations (*n*= 2 infants, *n*= 5 adults, *n*= 2 older adults) (Vedhara et al., 2019). In this review, a range of psychological interventions were assessed to examine their ability to act on vaccinations responses (influenza, hepatitis B, diphtheria, tetanus and pertussis), measured via antibody measures, to protect against disease. Although a modest number of studies were identified in this review, they displayed considerable heterogeneity in terms of intervention type, intervention duration, vaccination type and approach to assessing antibody responses to vaccination. Types of intervention included writing interventions (Petrie et al., 1995; Stetler et al., 2006), massages (Hsu et al., 1995; Huang & Huang, 1999; Loft et al., 2012), cognitive-behavioural stress management (Vedhara et al., 2003) and mindfulness meditation (Davidson et al., 2003; Hayney et al., 2014; Y. Yang et al., 2008).

One of the studies presented in this review investigated a body massage intervention lasting 45-minutes once a week for a total of 4-weeks (Loft et al., 2012). The intent of this intervention was to relax and de-stress a group of students before an examination period and to determine whether this had an influence on hepatitis B vaccination responses. In this study, Loft et al. (2012) examined whether a 4-week massage intervention could enhance antibody responses to hepatitis B vaccination and found it attenuated emotional stress and resulted in significantly lower antibody titres at 2-weeks and 6-weeks post-vaccination. In addition to full body messages, relaxation can also be achieved via mindfulness meditation practice as evidenced by Davidson et al. (2003) who assessed the effect of an 8-week clinical training program in mindfulness meditation to be applied in a work environment with healthy employees prior to influenza vaccination. Brain electrical activity was assessed and identified significant increases in left-side anterior activation associated with positive affect observed in the mindfulness meditation group compared with controls. The meditation

group also had significantly higher antibody titres to influenza vaccination, whereby the rise in antibody titre was predicted by the magnitude of left-side anterior activation increase. This study demonstrated the ability of a mindfulness meditation interventions to modulate both brain and immunological changes to act positively on vaccination responses. This research is supported by Yang et al. (2008) who assessed whether 5-months of moderate Tai Chi, which incorporates exercise, relaxation and meditation into a single intervention, could improve immune responses to influenza vaccination in older adults. The intervention group had significantly higher antibody titres in both magnitude and duration at 3-weeks and 20-weeks, but not at 6-weeks compared with controls. However, no significant differences between groups in seroprotection rates (4-fold increase in antibody titres) were observed. Research consistent with these findings was conducted by Hayney et al. (2014) who were unable to enhance immune responses to influenza vaccination in adults using a mindfulness-based stress reduction intervention. Despite no significant improvement in antibody responses to vaccination, the overall seroprotection rates (defined as >160 hemagglutination units) to any of the vaccination components was 96%. This was considered to be an extremely robust immune response shown by most participants and may be the reason statistical differences in immune responses could not be shown between intervention and control groups. Interestingly, these participants considered to be seroprotected reported higher levels of optimism, less anxiety and lower levels of perceived stress compared with non-responders. Another explanation for the lack of group differences could be the study protocol not including a measure of mindfulness meditation adherence beyond class attendance. Additionally, the inclusion criteria of this study meant that only participants without prior interest in meditative practice could be recruited. This is a limitation due to the known value of intervention belief and engagement for enhancing its effectiveness (Sekhon et al., 2017).

In contrast to the above findings, Vedhara et al. (2003) examined whether an 8-week stress management intervention could enhance responses to influenza vaccination in elderly caregivers and found that significantly more caregivers in the intervention group were classed as seroprotected compared with caregivers in the control group. However, seroprotection rates did not differ significantly between intervention caregivers and non-caregiver controls, and significantly more non-caregiver controls were classed as seroprotected compared with caregiver controls. This evidence suggests that mindfulness meditation and stress management interventions have the potential to alter antibody

responses to vaccination in older adults but the mechanisms and the extent to which they can impact meaningful on clinical outcomes is unclear.

The above-mentioned studies have only taken into account antibody responses to vaccination, but have neglected the important cellular-immunity component of the immune system. Particularly in older adults, it is important to assess immune responses as a whole by also taking into consideration lymphocyte, monocyte and cytokine responses to vaccination to better understand the clinical implications of psychological interventions (McElhaney et al., 2006). Evidence has indicated that antibody responses to vaccination, whilst conventional, were not the most accurate measure to determine disease protection, with CD4+ T cell responses sometimes serving as better correlates of protection (Plotkin, 2010). Studies that reported laboratory confirmed disease were more accurate for correlating disease but were not commonly used in the context of psychological interventions due the requirement for longer follow-ups and increased costs (Parikh et al., 2016). It was also not reasonable to suggest that increases in absolute antibody levels translated into improved rates of seroprotection, especially in the case of studies that report improvement in only one immune outcome instead of all immune outcomes. For example, Stetler et al. (2006) showed evidence of improved antibody responses for a writing intervention group compared with controls, but only did so for one of the three vaccination components. The data for the remaining two vaccine components were not presented in the manuscript so cannot be considered. However, there is a study by Irwin et al. (2007) who examined whether Tai Chi (gentle physical exercise and stretching as a form of meditation) could alter vaccination responses by assessing T cells. This study found that older adults trained in Tai Chi have significantly improved responses to varicella zoster vaccination as measured by T cell responses. This suggested that perhaps an explanation of the lack of statistical differences observed in the study by Hayney et al. (2014), as well as other intervention studies that only measure antibody immunity, was due to the lack of cellular immunity measures rather than there being no immune modulation differences occurring between intervention and control groups. This suggested that there may still be value in the utilisation of mindfulness mediation to improve antibody responses to vaccination, however more research needs to be done to investigate cellular immune responses and to determine whether these types of psychological interventions are able to impact meaningfully on clinical outcomes.

On balance, the majority of the evidence presented above did offer some support for the utilisation of psychological interventions as vaccine adjuvants. It also appeared that

intervention effects were generalisable across a range of vaccinations, which was evidenced by the fact that intervention effects are unrelated to vaccination type or timing of the intervention relative to vaccination administration. The exception would be in the context of vaccinations that are particularly immunogenic, like observed in the study by Hayney et al. (2014), that show very high vaccine efficacy. However, the evidence also presented some important issues and limitations that needed to be considered. Whilst interventions may show promise in improving immune responses to vaccination, it is unclear whether one of these types of intervention is the most influential due to the lack of literature and broad range of intervention types reviewed. It is also of critical importance for studies investigating the influence of psychological interventions on vaccination responses to examine more immunological outcomes, such as T cells and cytokines, rather than only antibody outcomes to better understand the biological mechanisms altered. The evidence also presented practicality issues due to the high cost and duration of these interventions, where in some instances professional equipment or expertise are required. As mentioned earlier in this section, duration of psychological interventions within the context of clinical environments is an important consideration for determining whether intervention implementation is feasible. Brief interventions (defined as single-session) have been trialled and reviewed to induce positive mood and could be more a more feasible option to explore in the context of clinical settings (Ayling et al., 2020). The reason for this is because pragmatically brief interventions are the best option to implement alongside routine vaccination care due to the practical and economic challenges of implementing long and complex interventions in clinical settings.

#### *2.6.2.2 Brief Interventions and Immunity*

Similar to non-brief intervention studies, brief-interventions have also explored massage as a method of reducing anxiety and improving immune responses (Donoyama et al., 2010; Donoyama & Shibasaki, 2010; Groer et al., 1994; Noto et al., 2010). In all of these massage intervention studies, salivary secretory immunoglobulin A (IgA) was the immunological outcome measure, whereby secretory IgA concentrations were shown to increase significantly for all groups (intervention and controls). Groer et al. (1994) was the only study that demonstrated a significant difference in secretory IgA concentrations between intervention and non- intervention groups and was the only one to measure secretory IgA secretion rate, which was also increased for both groups but did not significantly differ between groups. No significant differences in secretory IgA concentrations were observed between groups for the remaining massage intervention studies. However, most studies

were able to demonstrate a significant decrease in anxiety scores for participants allocated to the massage intervention group (Noto et al., 2010).

In contrast, two other studies focused specifically on massage therapy at the vaccination injection site as opposed to a full body massages outlined in the non-brief intervention section (Hsu et al., 1995; Huang & Huang, 1999). In both of these massage studies, the focus was to examine the effect of local massage on adverse reactions and immunogenicity of the diphtheria-tetanus-pertussis vaccine. Hsu et al. (1995) found that a post-vaccination brief intervention of 1-minute light circular massaging over the vaccination injection site resulted in significantly higher diphtheria and pertussis antibody titres at 2-months compared with the no intervention control group. This local massage intervention was associated with better immunogenicity and more adverse reactions, including low grade fever and mild local pain. Huang and Huang (1999) increased the intensity of this local massage intervention by increasing the duration to 2-minutes (doubling the time) and also incorporating the application of a warm towel at the injection site for 30-minutes in the evening of vaccination day. Researchers found that despite an increase in massage intensity, there were no significant intervention and control group differences in antibody titres for diphtheria, tetanus or pertussis. A possible explanation could be due to the increased intensity of the local massage being too vigorous resulting in increased adverse reactions at the site of vaccination with no beneficial effect on antibody response. One of these studies was able to demonstrate the immunological benefits of a massage intervention on vaccination responses but raises the question about whether or not this intervention type can be classified as or considered to be a psychological intervention. In both of these studies, the aim was to deliver a local massage at the site of injection, whereby the rationale behind the development was not psychological but was instead as a method of promoting immune activity via the disruption of tissue activity to aid absorption of the vaccine.

The above evidence suggested that the brief massage intervention did not appear to have significant immunological benefits, thus contrasting the evidence outlined for non-brief massage intervention studies. Whilst both brief and non-brief massage interventions were capable of beneficial psychological effects by reducing anxiety, it appeared that this particular intervention type needed to be increased in duration and in frequency to be able to influence immune responses. However, this would be practically challenging due to resource, time and costing restraints that would make a longer lasting massage intervention impossible to implement within clinical settings, including standard vaccination clinics.

A particularly interesting intervention type referenced in the literature was that of odour to either evoke autobiographical memory or expose individuals to pleasant odours (e.g., lemon, lavender) (Kiecolt-Glaser et al., 2008; Matsunaga et al., 2011). The phenomenon of certain smells evoking specific memories has been well researched and is known as the Proust phenomenon (Matsunaga et al., 2011). A variety of studies have indicated that odour-evoked autobiographic memory accompanied by positive emotions were capable of having psychological and physiological effects, including brain-immune interactions. Matsunaga et al. (2013) found that participants exposed to nostalgic odours experienced positive emotions and autobiographic memories, whereby levels of pro-inflammatory cytokines (i.e., TNF-α and IFN-γ) were significantly reduced. Brain imaging (Positron Emission Tomography images) found that certain areas of the brain (orbitofrontal cortex and posterior cingulate cortex) were significantly activated during experiences of odour-evoked autobiographic memory, whereby the activity of these brain areas were negatively correlated with IFN-γ concentrations. Matsunaga et al. (2011) found similar results when exposing participants to smells that were nostalgic to them, with increased positive mood states (e.g., comfort and happiness) and decreased negative mood states (e.g., anxiety). Researchers also noted that the odour group displayed a significant decrease in heart rate and peripheral IL-2 levels compared with controls. Other research has also focused on more generic smells, by exposing participants to pleasant odours that are classified by aromatherapy as being relaxing (lavender) and stimulating (lemon) (Kiecolt-Glaser et al., 2008). Lemon oil for 75-minutes was shown to reliably enhance positive mood, compared with lavender and water (control group). None of the odours reliably altered IL-6 and IL-10 production, salivary cortisol or heart rate. This evidence suggested that this intervention type could be a promising avenue to explore in the context of vaccination, where it would be interesting to examine whether the brain-immune responses to odour translate into improvements in vaccine-specific antibody and/or T cell responses. A potential issue odour-interventions could present within clinical settings could be difficulty in personalising odours that are nostalgic for each individual. This could be overcome by selecting more generic, pleasant odours as seen by Kiecolt-Glaser et al. (2008), such as lemon. However, the lack of evidence of odour influencing physiological outcomes, such as heart rate and immune measures, and the 75-minute timeframe could make this a less desirable avenue to explore for future research aiming to administer interventions within clinical settings.

Comedy, film, audiotape and music was the commonly referenced intervention type in the literature, perhaps because it offered the ability to easily alter intervention duration, content

and did not require expert training or equipment to develop or deliver (Ayling et al., 2019; Bennett et al., 2003; Kimata, 2004; Koyama et al., 2009; Labott et al., 1990; Matsunaga et al., 2008; Mittwoch-Jaffe et al., 1995; Takahashi et al., 2001). This intervention type was shown to have beneficial psychological effects including significantly decreased stress levels (Bartlett et al., 1993; Bennett et al., 2003; Rider et al., 1990), increased laughter expression (Koyama et al., 2009; Labott et al., 1990) and increased positive mood (Ayling et al., 2019). Interestingly, Rider et al. (1990) noted the importance of musical preference when developing a music-based intervention to improve intervention effectiveness. This research was consistent with previous evidence that suggested that choice interventions, such as music preference selection, had the potential to increase intervention engagement and effectiveness. Giving participants an element of choice was shown to lead to increased interest and motivation, where having the choice gave participants feelings of personal control (Cordova & Lepper, 1996). It is no surprise that people were receptive to choice because it has been engrained in our society that restriction or elimination of choice makes people feel like they have lost an element of control that can lead to negative behaviours, such as aggression (Adelinis, 2005).

Although there is evidence of a beneficial psychological impact of these interventions, they have varying influences on immune responses. Bartlett et al. (1993) for example found no significant differences in NK cell cytotoxicity change between participants who viewed a comedy film compared with those who did not. Similarly, Ayling et al. (2019) developed a brief 15-minute intervention containing high arousal stimuli in multiple forms (e.g., uplifting music, images, comedy) to appeal to different tastes specific to older adults. Despite significant increases in positive mood (assessed on a visual analogue scale of happiness (Kontou et al., 2012)) for the intervention group compared with control group, no significant differences in antigen-specific IgG responses to influenza vaccination were reported. However, point estimates of effect size did favour participants in the intervention group at both 4-weeks and 16-weeks post-vaccination suggesting some immunological potential. This study also focused solely on antibody responses to vaccination and disregarded other important components of the immune system that could be altered. Koyama et al. (2009) explored recreational music-making modulation of mood states and immunological responses in older adults and found a significant increase in the number of lymphocytes, T cells, CD4+ T cells, memory T cells and the production of IFNγ and IL-6. As mentioned earlier in this chapter, some research has evaluated Th1 and Th2 cytokine ratios of IFNγ:IL-10 and found that chronically stressed older adults underproduced IFNγ and overproduced IL-10

reflecting a shift towards a Th2 response (an anti-inflammatory, immunosuppressive response) (McElhaney et al., 2006). Koyama et al. (2009), on the other hand, found a shift opposite to that of stress with an increase in IFNγ and unchanged IL-10 reflecting a Th1 response (pro-inflammatory response) in the group of older adults who had their mood improved. This was another example that highlighted the importance of exploring more than antibody immunity in older adult populations to create a broader picture of the potential immune changes that could be occurring. Other evidence has indicated significantly increased IL-2 and IL-3 levels and significantly decreased TNF-α levels from before to immediately after comedy film intervention (Mittwoch-Jaffe et al., 1995), and significantly increased NK cell activity (Takahashi et al., 2001). Bartlett et al. (1993) also found that participants who listened to music significantly decreased cortisol levels and also produced a significant increase in IL-1 levels, with the control groups showing no significant change for either of these outcome measures. The majority of the evidence presented above was suggestive of a relationship between comedy and music interventions and improved psychological and immunological outcomes.

It is also worth noting that there has been a considerable amount of research that investigated the influence of short bouts of physical activity interventions on the enhancement of positive mood (Müller et al., 2021) and immune outcomes (Nieman & Wentz, 2019; Walsh et al., 2011). For example, Muller et al. (2021) conducted two studies to determine whether mindfulness and physical activity played a role in the induction of positive mood and found that short bouts of either was beneficial in increasing mood scores compared with controls. Short bouts of physical activity (60-minutes or less) has also been shown to alter immune responses by upregulating NK cells and CD8+ T cells (cytotoxic immune response), as well as anti-inflammatory cytokines, neutrophils and immature B cells (Bigley et al., 2014; Nieman et al., 2005). The confounding effects of physical exertion (e.g., increased bodily movement and respiration) make it difficult to determine the relationship between positive mood changes and immunity. It may be that physical exercise is similar to that of local massages at the vaccination site (see *Section 2.6.2.1*), where it acts directly on the immune system and is not necessarily considered to be a psychological intervention. Regardless of this, there still may be merit in utilising physical exercise for mood and immune improvement but this may also only be applicable to younger adults as opposed to older adults. Tasking older adults with short bouts of moderate to high intensity exercise could be practically and ethically problematic due to the known frailty and common underlying health conditions of this age group. For this reason, it would be more beneficial for researchers

aiming to enhance the positive mood of older adults to adhere to less physically exerting interventions, such as music and comedy, that have shown some promise in inducing positive mood and immune responses but do not offer the issue of health concerns.

The evidence presented in this section provides modest support for a range of brief positive mood enhancing interventions acting positively on immune outcomes. Similar to the non-brief intervention evidence, the literature around brief interventions was considerably heterogeneous with no single type of intervention highlighted as being the most effective for altering mood and/or immune parameters. The large majority of interventions were shown to elicit a significant improvement from pre-intervention to post-intervention in at least one mood and one immunological outcome, whereby secretory IgA, NK cells and IL-6 were some of the most commonly referenced immune outcome measures. Secretory IgA was frequently measured because it has been demonstrated to be an effective short-term measure of immunocompetence. However, most studies measured short-term immunological responses by only measuring post-intervention immune outcomes instead of looking at follow-up responses. Looking at primary immune responses in isolation, whereby these are measures of non-specific immunity, makes it challenging to determine whether the immunological changes that occur are able to impact meaningfully on clinical outcomes.

Future research looking into follow-up immune responses could provide insight into the influence of brief interventions on secondary immune responses, which could be valuable in the context of investigating vaccination responses or evaluating clinical outcomes. Researchers should consider examining a broader range of immunological measures in their studies to incorporate both cellular and humoral immune outcomes, such as T cell and antibody responses, to be able to better understand the immunological effects of psychological interventions.

### *2.6.3 Thesis Rationale*

This section has reviewed some key brief and non-brief psychological intervention types and their influence on an array of immune responses, from secretory IgA concentrations and antibody responses to cytokine regulation. Several studies have examined brief interventions lasting 60-minutes or longer, however, in the context of exploring interventional (non-pharmaceutical) avenues for improving vaccination responses in older adults, it is important for intervention duration, resource and cost to be taken into account. Non-brief intervention types, such as a full body massage of 45-minutes once a week for a total of 4-weeks, as demonstrated by Loft et al. (2012), was shown to attenuate stress and significantly alter

antibody responses. Although massage interventions for relaxation and stress could be a promising avenue, it is not a practical or feasible option due to the high cost and resource required (i.e., specialist masseurs and masseuses). Many of the other non-brief interventions have also used expensive equipment or resources (e.g., music equipment, trained professionals). For interventions that are looking to be incorporated into clinical settings, such as the application into standard vaccination care, the practicalities of intervention implementation are important to consider. Generally speaking, clinical settings are inundated with patients and are severely short-staffed meaning that any interference with the standard of care should be as easy and/or brief as possible.

In contrast, some brief intervention types (e.g., films and listening to music) would be much easier to implement within clinical settings than others (e.g., massage, music making), making these intervention types more desirable to be implementation within standard care, such as within vaccination clinics. From a resource perspective, they would also be less costly and would not require skilled or trained professionals. Some interventions lasting 15-minutes or less were shown to be promising in terms of immunological evidence and have greater potential for utilisation within clinical settings (e.g., comedy ad music). A key example of this is the study by Ayling et al. (2019) who explored whether a brief intervention, 15-minutes of comedy and music delivered to older adults (*n*= 103) on a tablet device, could enhance positive mood and improve antibody responses to influenza vaccination. Compared with a neutral intervention (time-matched), this comedy and music intervention was found to significantly enhance the positive mood of older adults, but no significant difference in post-vaccination antibody responses were reported. Whilst these findings were unable to demonstrate any immunological impact, the study was only powered to be able to detect a between group change in positive mood and not in antibody responses. However, point estimates of effect size did favour participants in the intervention group at both 4-weeks and 16-weeks post-vaccination suggesting some immunological potential. Feedback from this study found that the implementation of a tablet device-based intervention within a standard vaccination clinic was relatively straight forward, with only some technical issues due to the nature of the study cohort being older adults and were therefore less familiar with using the technology. Ultimately, this study played a crucial role in the direction of the research conducted as part of this thesis, whereby the findings were utilised to contribute towards the development of a larger scale study to investigate the role of uplifting music, images and comedy on improving the positive mood of older adults within the context of standard vaccination care. Herein lies the focus of this thesis: investigating the implementation of two

brief psychological interventions (standardised and choice, described in more detail in the following chapter) to improve the positive affect of older adults prior to influenza vaccination. More details about the design and methods of this research are outlined in *Chapter 3*.

## 2.7 Chapter Summary

In this chapter, the reader was introduced to the body of evidence that surrounds the influence of negative and positive emotional experience on immune responses with a particular focus on vaccination where possible. Chronic stress was highlighted to be an important immunosuppressive psychological factor, whereas positive mood was demonstrated to have immunoenhancing effects. The literature highlighted psychological interventions as a potential avenue for improving psychological and immunological outcomes, whereby positive mood on the day of vaccination was associated with enhanced antibody responses to some strains of the influenza vaccination in older adults. An important intervention example, that has played a key role is shaping the research direction of this thesis, has shown that a brief intervention consisting of comedy, film and music is capable of enhancing the positive mood of older adults prior to influenza vaccination. To build on this research, the aim of the research presented within this thesis was to conduct a larger scale study (referred to as the For-ME trial) to explore whether music and comedy-based interventions were capable of improving the positive mood of older adults and consequently improve antibody responses to vaccination. In *Chapter 3*, the Flu and Mood in Older Adults (For-ME) randomised control trial design and methodology is described in detail.

# 3 Chapter 3: The For-Me Trial Design and Methods

## 3.1 Chapter Introduction

As described in *Chapter 1*, pharmaceutical avenues for improving vaccination responses have been explored but have not yet been shown to improve the immune responses of older adults (≥65 years old) to the same degree observed in young adults. This has highlighted the need for an alternative method to improve vaccine effectiveness for this vulnerable population (McKee et al., 2007).

Interventions targeting psychological factors to improve vaccine responses have shown some promise (see *Chapter 2*). Recent observational research sought to determine the respective contribution of different psychological (stress, positive affect, negative affect) and behavioural (physical activity, nutrition, sleep) influences on influenza vaccine responses in older adults (Ayling et al., 2018). This study identified positive affect, on the day of vaccination, to be the most significant factor for predicting 4-week and 16-week antibody responses to vaccination, where it was associated with enhanced responses to influenza vaccination strain A(H1N1).

Following this research, a pilot trial was conducted to test a brief positive mood intervention administered on the day of influenza vaccination in older adults (Ayling et al., 2019). This pilot trial showed, compared with a neutral mood intervention, that the positive mood intervention significantly improved mood. However, no statistical differences of group allocation on antibody responses to vaccination were reported. It is important to note that this trial was not powered to detect a difference in antibody levels between groups. However, the point-estimates favoured the intervention group in terms of antigen-specific IgG responses to influenza vaccination.

The Flu and Mood in Older Adults (For-ME) trial, which forms the bulk of research presented in this thesis, builds upon this foundation of research. The trial primary aim was to investigate whether a standardised and/or choice intervention, compared with usual care, were capable of improving positive mood on the day of influenza vaccination in older adults and to identify which intervention type was superior. The trial secondary aim was then to examine whether group allocation (standardised intervention, choice intervention or usual care) could predict 4-week antibody responses to vaccination. This chapter describes the design, development and methodology of this 3-armed, double-blind, randomised control trial (RCT) conducted between August 2019 to May 2020. This trial's research governance and ethical approval was given by the Health Research Authority and East Midlands-Nottingham Research Ethics

Committee 1 (19/EM/0081) prior to trial commencement. The trial was preregistered at clinicaltrials.gov (NCT03956329).

## 3.2 Initial Considerations

### *3.2.1 Trial Design*

Observational studies are where both exposures and outcomes are observed, and researchers do not influence participants. Conversely, experimental studies aim to investigate a particular intervention or treatment, whereby participants are assigned to different groups and outcomes are observed. Using an experimental study design, particularly a randomised control trial, allows for the direct comparison of a measurement of the outcome's effect against a stable control. This gives a researcher a degree of certainty about whether the intervention is responsible for any observed changes in outcome. Whilst being considered the gold standard for measuring the efficacy of a treatment (Barton, 2000), the rigorous methodology of a randomised control trial (RCT) also eliminates bias relating to confounding factors (by having a control group), selection bias (via randomisation) and interpretation bias (by single or double-blinding) (Ernest et al., 2015). For this reason, it was deemed a RCT design was most suitable for the For-ME trial in order to compare two different intervention types (i.e., standardised and choice) with a control condition. The next consideration was to decide on the type of control condition to be used.

#### *3.2.1.1 Choosing an Appropriate Comparator Group*

A consideration of the For-ME trial was to determine whether the two positive mood interventions should be compared to a neutral control video, or whether the trial control should more closely replicate usual vaccination care. In the previous pilot trial that examined a positive mood intervention compared with a neutral intervention, some participants found the neutral intervention enjoyable and reported an increase in positive mood (Ayling et al., 2019). This highlighted that a neutral intervention was not a sufficient control condition, due to it unintentionally increasing participant positive mood, and highlighted the need for an alternative. A possible alternative control condition could be the implementation of a usual care condition, that closely replicates usual vaccination care, that would involve participants sitting in a waiting room for 15-minutes (the same duration of time that participants engage with an intervention) prior to vaccination. This also presented some difficulties due to the nature of general practice (GP) waiting rooms often playing background music and leaflet and television advertisements that have the potential to influence positive mood. This is a challenge because it would be difficult to control these factors between different general

practice (GP) surgery locations (a total of 13 GP surgeries were recruited and took part in the For-ME trial).

On balance, limit the exposure of participants to external factors, such as background music, participants were instructed to sit in a study room instead of a waiting room. The benefit of having a usual care control arm means that both positive mood interventions are more closely compared to current vaccination care and procedure, and can therefore better indicate whether one or both of the positive affect interventions are superior to usual care.

### *3.2.2 Vaccination Choice*

Whilst there is a plethora of research investigating the behavioural and psychological influences on vaccination, including Hepatitis B (Marsland et al., 2006; Prather et al., 2012), Keyhole Limpet Hemocyanin (Grant et al., 2008) and Pneumococcus (Glaser et al., 2000), most studies have opted to investigate influenza vaccination responses for older adult populations (Kiecolt-Glaser et al., 1996; Moynihan et al., 2004). One of the reasons for this is because older adult populations have a much lower influenza vaccine efficacy rate, at an estimated 20-50% compared to younger adults at around 70% (Lang et al., 2012). Annually, over 7 million influenza vaccinations are administered to older adults, making it the most received vaccination for that population (Public Health England, 2018). Also, conducting a trial that involves influenza vaccine administration allows for the ability to 'piggyback' onto existing vaccination schedules implemented by GP surgeries. This is a big strength when running a trial because it raises fewer ethical concerns due to the vaccination program forming part of the standard of care for participants taking part. However, influenza vaccination trials also come with a disadvantage due to the large annual vaccine uptake interfering with pre-vaccination antibody levels, especially in repeated strains that are commonly observed for influenza. In the case of vaccinations that are administered annually, especially those with repeated strains, individuals can present high levels of pre-vaccination antibodies, which can make it challenging to assess antibody responses to vaccination. This is because in some cases participants are also classified as responders (or seroprotected) pre-vaccination and means that the assessment of change in antibody levels from pre- to post-vaccination would be inaccurate.

Nonetheless, the advantage of being able to run the trial in conjunction with an established vaccine program outweighed this disadvantage and so the decision was made to focus on influenza vaccination, where the challenges of dealing with influenza vaccination are managed and described later in this chapter. Following this decision, the specific type of

vaccination for older adults needed to be selected. Contrary to most years, two different influenza vaccinations for older adults (≥ 65 years) were approved for the 2019/20 influenza vaccination season by NHS England. These two influenza vaccinations included the adjuvanted trivalent injected vaccine (aTIV) (commercially known as Fluad) (grown in eggs) and the quadrivalent injected vaccine (QIVc) (cell-grown), where both were considered to be equally suitable (National Health Service, 2019).

On a purely scientific basis, selecting a non-adjuvanted vaccine was most appealing when considering the trial secondary aim of measuring antibody responses to vaccination. Adjuvants are added to vaccinations because they contain substances that boost an antigen's immunogenicity and, therefore accelerate vaccine responses. Depending on the type of adjuvant used, for which there is a broad spectrum to choose from, the quality of an immune response to vaccination can vary significantly. Different adjuvant types can change cytokine and chemokine profiles leading to the recruitment of different immune cells, and therefore have a downstream influence on antibody responses (McKee et al., 2007; Principi & Esposito, 2012). Therefore, adjuvanted vaccinations add another dimension to consider when looking at immunological responses to vaccination (Tregoning et al., 2018). As a result, this made the use of the QIVc more desirable. However, this vaccine was also shown to be fractionally more expensive for clinical practices to purchase (£9.94 compared to £9.79 for the aTIV) (Powis, 2019). As a result, we discovered that most of the clinical practices recruited for the trial had opted for the cheaper option (aTIV). Including both types of vaccination would add additional variability, which would also make addressing the trial secondary aim of assessing vaccination specific antibody responses challenging.

Thus, primarily due to pragmatic reasons, it was decided that this trial would focus on using the aTIV vaccine suspension for injection 0.5ml pre-filled syringes (Seqirus Vaccines Ltd) (NICE, 2019). The decision to select the aTIV was primarily driven by the need to recruit a substantial sample of clinical practices (*n*= 13) and participants (*n*= 614). Choosing the QIVc would make recruitment of sufficient numbers too challenging, due to a smaller number of clinical practices administering this specific vaccine type, and would present a higher risk of not obtaining the desired number of clinics and participants for the trial.

### *3.2.3 Trial Length*

#### *3.2.3.1 Antibody Responses to Vaccination*

One key consideration that needed to be taken into account for determining trial length was post- vaccination antibody responses. When measuring antibody responses to vaccination,

most studies have looked into short-term antibody responses, which are typically measured 4-weeks post-vaccination and is when prior research has indicated that peak IgG antibody levels are reached (Gross et al., 1997; Siegrist, 2013). For this reason, it was determined that two blood samples would be obtained: one immediately prior to vaccination, as a baseline (pre-vaccination) measure, and one 4-weeks post-vaccination. The final 4-weeks post-vaccination blood sample was collected in December 2019.

### *3.2.4 Capturing State Affect*

The trial primary aim was to investigate whether a standardised intervention and/or choice intervention were capable of enhancing positive mood immediately prior to influenza vaccination in older adults, compared with usual care. It was therefore important to considered how to measure positive mood before and after intervention engagement, or sitting in the waiting room in the case of usual care, to test the primary aim. When referring to positive mood before and after intervention engagement or usual care, we are interested in the state affect of participants. It is important to highlight that state affect refers to mood in the present moment (e.g., before and after intervention engagement), which differs from trait affect that refers to general mood over longer periods (e.g., months) (Hur et al., 2015).

When measuring state affect and determining whether an intervention has influenced state affect, researchers often use self-report measures. Positive and Negative Affect Schedule (PANAS) (Watson et al., 1988) is considered one of the most established methods (Harmon-Jones et al., 2016). The PANAS scale was implemented in the previous pilot trial investigating a positive mood intervention in older adults, however the researchers found the PANAS scale was insensitive to change compared with alternative self-report measures, such as the Affective Slider scale (Ayling et al., 2019). Despite the face validity of self-report measures, literature has highlighted the dismissal of emotion in some instances whereby no increase or decrease in positive or negative affect was found with the PANAS scale. However, increasing knowledge in the field has indicated this to be flawed logic and has suggested self-report measures only measure a modest portion of affect cognition, therefore implying researchers prematurely discount emotion by only measuring conscious emotional processing by using the PANAS scale (Harmon-Jones et al., 2016; Mauss & Robinson, 2009). For these reasons, the decision was made to not use the PANAS scale as a measure of state affect, and therefore alternative measures needed to be explored.

To measure as broad a spectrum of state affect as possible, this trial implemented the use of multiple state affect measures, including: Affective Slider (happiness and alertness

subscales), Dynamic Visual Analogue Scales (DVAMS) (happiness and alertness subscales) and Scale of Positive and Negative Experience (SPANE). These are described in more details below.

The Affective Slider scale is a self-report measure that is a reliable, valid and sensitive method of measuring state affect (Betella & Verschure, 2016). The decision was made to incorporate the Affective Slider self-report measure into this trial because it provides researchers with data across a continuum of values. This allows for the ability to better distinguish between pre- and post-intervention affect measures due to the highly sensitive nature of this affect measure. In addition, this scale has also been shown to be a sensitive measure of affect by Ayling et al. (2019) who used the happiness (Happy-Sad) and alertness (Sleepy-Alert) Affective Slider subscales to measure state affect pre-and post-intervention. It is important to note that whilst there are 3 different measures of state affect being implemented to assess pre- and post-intervention mood, the Affective Slider happiness subscale was considered to be the primary outcome measure. The Affective Slider alertness subscale and the two measures described below (i.e., SPANE and DVAMS) were therefore secondary outcome measures.

The second affect measure incorporated into this trial to measure pre- and post-intervention state affect was the Scale of Positive and Negative Experience (SPANE) (Corno et al., 2016; du Plessis & Guse, 2017; Li et al., 2013). The SPANE is a validated affect measure that has been shown to have benefits over earlier developed scales due to its ability to assess a broad range of positive and negative experiences and feelings instead of a specific type. It has good internal reliability when providing researchers with clinically useful information about the patient emotional experience and has therefore been deemed a good affect measure, due to it being sensitive to change, to be implemented into this trial (Barrows & Thomas, 2016).

The final affect measure used in the trial were the Dynamic Visual Analogue Mood Scales (DVAMS) (Barrows & Thomas, 2017). These measure were initially developed to assess the mood of stroke patients with aphasia, a condition where patients experience impaired spoken or written communication. This non-verbal, validated, self-report affect measure has been highlighted to enable patients to quickly and accurately report a profile of their mood and was also deemed suitable to be used as part of this trial (Quirin et al., 2009). The two types of DVAMS incorporated into the trial were the happiness and alertness subscales.

This trial was unable to account for unconscious emotional processing and therefore focused on conscious emotional processing in the form of self-report affect measures. There are very

few unconscious affect measures, where the Implicit Positive and Negative Affect Test (IPANAT) is most commonly cited (Tomlinson et al., 2019). On balance, it was decided that this trial would only focus on measuring affect using self-report measures (SPANE, AS and DVAMS). This was decided mainly due to Patient and Public Involvement (PPI) feedback, where older adults had indicated the use of the IPANAT to be extremely undesirable due to the lengthy and repetitive nature of the measure. Some participants had even expressed they would drop out of the study if they were required to continuously complete it due to the measure's repetitive nature (Ayling et al., 2018).

### *3.2.5 Inclusion and Exclusion Criteria*

Research indicated that influenza vaccination history and previous exposure to influenza viruses influences how well older adults respond to influenza vaccination (Public Health England, 2019a). This is largely due to influenza vaccines being very similar each year, and therefore individuals with prior influenza exposure would already have an immune repertoire to be able to better combat infection and to respond better to vaccination (Sasaki et al., 2008). Due to the nature of the target population of this trial being older adults, it is very likely they would have had previous influenza exposure either naturally or via previous influenza vaccination. To make previous exposure as consistent as possible across the participant cohort, only those who had received the previous years (2018/19) influenza vaccination were eligible to take part in the trial. Although the option of including older adults without influenza vaccination history was considered, controlling for this in statistical models would prove challenging. This is because it would be difficult to determine whether these participants have had previous natural exposure to influenza viruses. In addition, over 70% of older adults typically receive the influenza vaccination each year, therefore increasing the generalisability of findings by including older adults who have previous influenza vaccination history (Public Health England, 2019a). Although the option of only recruiting those who had been vaccinated the previous year (2018/19) only partially accounts for previous exposure, it ensures that all participants have had at least one prior exposure to the same influenza viral strains.

Research studies investigating vaccination outcomes in older adults have been known to use the SENIEUR protocol, whereby these studies aim to exclude older adults that display any sign of disease, frailty or non-standard immune functioning (Ligthart et al., 1984; Trzonkowski et al., 2009). As a result, SENIEUR protocol studies only include a narrow portion of the older adult population due to the nature of this population experiencing a high frequency of disease and frailty (Edelman et al., 2020; Ligthart et al., 1984). Research

investigating influenza vaccine efficacy in older adult populations has suggested that SENIEUR-compatible patients mount immune responses to vaccination, whereas non-SENIEUR patients do not respond as well to vaccination (Trzonkowski et al., 2009). Thus, highlighting the degree to which older adults with pre-existing medical conditions display suboptimal immune functioning. However, implementing the SENIEUR protocol would mean that only 15-20% of older adults meet the inclusion criteria of a trial, which is problematic because it limits the generalisability of findings and considerably narrows the participant recruitment pool (Penedo & Dahn, 2005).

As a result, the decision was made to include participants with disease and those on medication that may alter immunological responses to ensure the trial findings are representative and generalisable to the older adult population. However, participants whom a healthcare provider deemed to be too physically frail to participate and those diagnosed with dementia or other cognitive condition, which would make participation difficult, were excluded from the trial. In addition to this, participants were excluded if they were sufficiently hearing or vision impaired that meant exposure to the intervention as intended would be compromised.

### *3.2.6 Participant Burden*

Participant burden refers to any discomfort or inconveniences experienced by participants when taking part in a trial. In the case of this trial, participant burden included financial burden, due to each participant being required to attend their GP surgery for 2-visits throughout the duration of the trial. To ensure participants were not at a financial loss by taking part in this research, they received an inconvenience payment for each GP surgery visit they attended (£10 per visit).

### *3.2.7 Patient and Public Involvement*

Patient and public involvement (PPI) group insight is considered to be an important aspect of healthcare research and has been shown to improve research quality (Bedwell & Lavender, 2020; Patient, 2014). PPI group sessions allow patients to have a voice, where they are encouraged to share their views and input to help support research trial development and success. Incorporating PPI groups has been instrumental in providing researchers with the information needed to address better the relevance and impact of research for a specific patient group (Lewnard & Cobey, 2018; Sasaki et al., 2008).

Throughout the development process of this trial, 3 PPI group meetings were carried out with a group of older adults, recruited from a local community GP surgery, who spoke fluent

English and had no significant cognitive impairment. At the first meeting, the group was provided with an overview of what the trial would entail and were asked to provide feedback about the trial's strengths and weaknesses and whether this was research they themselves would consider taking part in. During the following two PPI sessions, the group also participated in various tasks, which included interactions with tablet devices to assess the pre- and post-intervention state affect measure methods and the positive mood interventions themselves. They provided valuable input about ease of use of the tablet devices and the quality of content provided within the interventions. The groups also addressed specific questions about the suitability of using the tablet devices regarding ease of reading and navigation, their preferred intervention content, the best method of contacting participants for the trial, and appropriate levels of inconvenience payments.

In sum, these PPI groups indicated this research purpose to be beneficial and useful for older adults and was something they would get involved in. PPI participants provided researchers with the following trial insights: the importance of maintaining regular contact with participants throughout the trial, and ensuring interactions with tablet devices and participant documentation (e.g., consent forms and baseline questionnaires) were simple and straightforward. Feedback indicated that tablet device alternatives needed to be provided for participants who were less comfortable or familiar with the use of tablet devices, and who may therefore find the experience of interacting with tablet devices challenging or stressful. As a result, the decision was made to implement paper copy completion of state affect measures (AS, DVAMS and SPANE) for participants who would prefer not to complete them using the tablet devices. It was also decided that a researcher would always be present whilst participants interacted with tablet devices to offer support and guidance when required. These considerations were of particular importance to ensure the trial primary aim, of examining whether a standardised intervention and/or choice intervention compared with usual care were capable of improving positive affect, was not negatively influenced by the potentially stressful experience of using a tablet device for this age group. They had also indicated that participant recruitment would be most suitable via a letter from their GP surgery. PPI participants had suggested excluding specific videos and music for the interventions, where the majority had enjoyed their tablet device experience. Additional minor amendments made following PPI involvement included: increasing tablet device text size and styluses to improve their tablet device interactivity and experience. Finally, the group helped identify the most appropriate inconvenience payment level to be £10 per GP surgery visit.

## 3.3 For-ME Trial Design

Following the above-mentioned initial considerations and PPI input, the final iteration of this trial was produced. A 3-arm, parallel, randomised control trial was conducted alongside the 2019/20 annual influenza vaccination schedule between August 2019 to May 2020. This trial's research governance and ethical approval was given by the Health Research Authority and East Midlands-Nottingham Research Ethics Committee 1 (19/EM/0081) prior to trial commencement (*Appendix A*). The trial was preregistered at clinicaltrials.gov (NCT03956329). This trial was also portfolio adopted by the Clinical Research Network.

## 3.4 Participants

Participants were recruited from 13 primary care GP surgeries within the East Midlands area. A summary of the flow of participants throughout the trial is outlined in *Chapter 5 "Results"*.

### *3.4.1 Inclusion and Exclusion Criteria*

To be eligible for inclusion in the trial, participants needed to be aged between 65 and 85 years old and were required to have received the influenza vaccination from the previous year (2018/19 season). Participants included were those eligible to receive the 2019/20 influenza vaccination as part of usual care. For informed consent to be taken, all participants with insufficient proficiency of the English language were excluded. Patients diagnosed with dementia or other cognitive conditions and who were deemed by a healthcare provider to be too physically frail to participate were excluded. It was also crucial for participants to interact with the interventions and questionnaires as intended, which excluded participants with sufficiently impaired hearing or vision. Participants who participated in the previous pilot trial conducted by Ayling et al. (2019) were also excluded from this trial. This is because there is a high chance that some participants from the previous pilot trial would have had prior standardised intervention exposure, whereby previous involvement would be a confounding factor that is difficult to control for and meant some participants would be unblinded. A summary of the trial inclusion and exclusion criteria is highlighted in *Table 3.1.*

**Table 3.1:** For-ME trial inclusion and exclusion criteria.

| **Inclusion Criteria** |
|---|
| • Males and females aged 65-85 years (inclusive) |
| • Received influenza vaccination for the 2018/19 season |
| • Eligible to receive 2019/20 influenza vaccination as part of usual care |
| • Ability to give informed consent |
| **Exclusion Criteria** |
| • Deemed by a healthcare provider to be too physically frail to participate |
| • Diagnosed with dementia or other cognitive condition which would make participation difficult |
| • Insufficient comment of English language |
| • Sufficiently impaired of hearing or vision that exposure to the intervention or control video content as intended would be compromised |
| • Those for whom the collection of blood samples is contraindicated |
| • Those who have participated previously in the pilot trial (Ayling et al., 2019) |

### *3.4.2 Sample Size and Randomisation*

The sample size was informed by the previous pilot trial (Ayling et al., 2019) and systematic review (Ayling et al., 2020) that looked into the effects of brief mood-improving interventions on mood outcomes. Based on these, a conservative medium effect size (d=0.4) was used for the power calculations. Therefore, calculations performed in GPower, determined that to detect a medium-sized (d=0.4) difference in the primary outcome between experimental arms and control with 90% power in separate one-tailed t-tests, a sample of 253 participants in each experimental arm and 108 participants in the control arm was required. For a smaller effect size, this will also give 80% power to detect a smaller (d=0.25) difference between experimental arms in a two-tailed t-test. To allow for reasonable attrition levels, the recruitment aim for this trial was set at 650 participants in total.

Participants were individually randomised on a 2:2:1 ratio to one of the two intervention arms (standardised or choice intervention) or to the usual care arm, respectively. The unequal randomisation ratio was implemented to have more power to be able to detect a difference between the two intervention arms. This is because, based on previous research, there was a high degree of confidence that a change between usual care and intervention(s) would be observed, however differences between intervention arms were predicted to be

much smaller. A third-party researcher carried out randomisation and participant allocation by generating a block randomisation sequence (block size= 10). This randomly generated sequence was paired to participant ID numbers in such a manner that touchscreen computer tablet devices were programmed to run the appropriate intervention/ usual care program without researchers being aware of participant intervention assignment. To avoid contamination, participants randomly allocated to the usual care arm were booked into separate sessions to those receiving one of the interventions. This meant that the researchers were unblinded to usual care allocation but remained blinded to which of the two intervention arms patients were allocated to. Researcher unblinding only occurred after patient-reported outcomes and blood samples were collected and analysed.

## 3.5 Procedure

Eligible participants received a trial information sheet (*Appendix B*), which included the trial requirements and a participation information letter from their GP surgery. This information pack contained an interest reply slip (*Appendix C*) and free post envelope for participants to return to researchers to express an interest in taking part. In some instances, participants expressed an interest directly via email or telephone using the provided researcher contact details within the information pack. Potential participants were contacted, whereby eligible participants were enrolled onto the trial and were randomised to a trial arm.

After initial enrolment, participants were posted a baseline questionnaire (*Appendix D*) to capture demographic information (age, gender, ethnicity, education, household income, marital status, living status and smoking status), health status and trait psychological factors (perceived stress and trait affect) (see below for more information). This questionnaire was returned to researchers via the use of a freepost envelope prior to their first scheduled GP surgery visit.

At the first scheduled GP surgery visit, participants completed written consent (*Appendix E)* to take part (*n*= 51 participants did not attend this session, or did not provide written consent, and were therefore removed from the trial). Typically, participants were booked into trial appointment groups of 5-7 people with each session lasting approximately 1-hour. Following consent (*n*= 654), participant height and weight was measured to determine body mass index and provided a venous blood sample (8mL) to determine pre-vaccination antibody status. Participants were then given a brief demonstration on how to use the computer tablet device (model: ASUS-T101HA, AsusTek Computer Inc.) and completed pre-intervention state affect measures (Affective Slider, DVAMS and SPANE) on the device.

Participants then received an intervention or usual care exposure (see below for more details) lasting a total of 15-minutes. Immediately following this, participants completed post- intervention affect measures (Affective Slider, DVAMS and SPANE) and were administered the 2019/20 season northern-hemisphere Fluad® Adjuvanted Trivalent Influenza Vaccine (aTIV) (Surface Antigen, Inactivated). Vaccine administration would typically be done less than 5-minutes following the end of the intervention or usual care exposure.

Exactly 4-week after the first visit, participants attended a second GP surgery appointment to provide post-vaccination blood samples (8mL) for determination of antibody responses to vaccination. At this visit, participants received an inconvenience payment of £20 (£10 for each GP surgery visit) for their time and to cover any travel expenses. An overview of the trial procedure is illustrated in *Figure 3.1.*

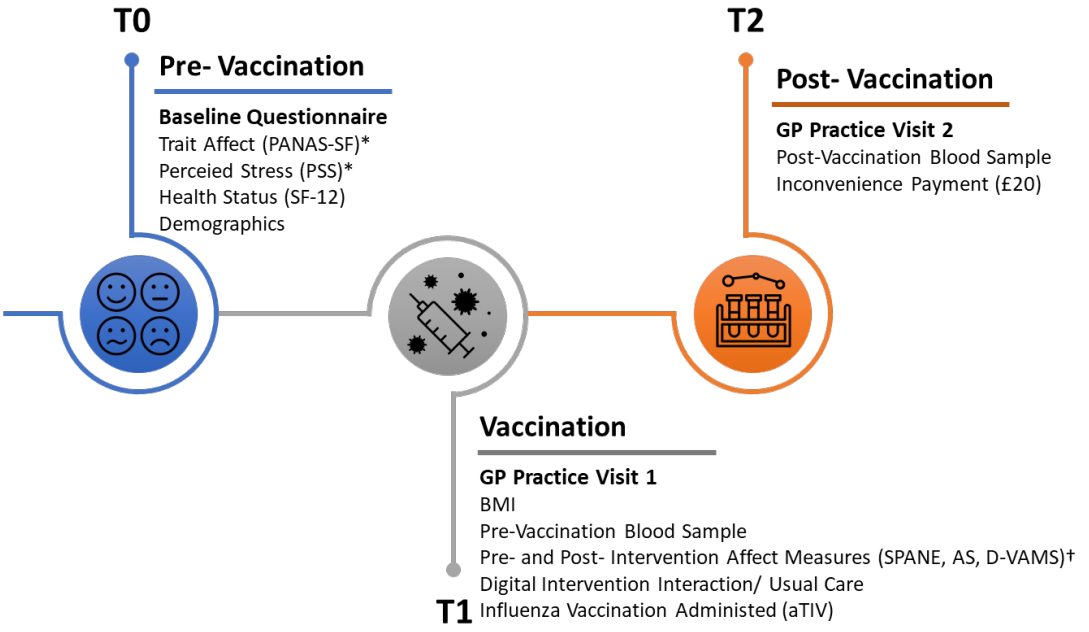



**Figure 3.1**: Overview of For-ME trial procedure.

PANAS= Positive and Negative Affect Schedule Short Form; PSS= Perceived Stress Scale; SF-12= 12-Item Short Form Survey; SPANE= Scale of Positive and Negative Experience; AS= Affective Slider; DVAMS= Dynamic Visual Analogue Mood Scales; aTIV= Adjuvanted Trivalent Injected Vaccine. (*) = Instructions require responses 'to what extent you generally feel this way'; (†) = Instructions require responses 'at the present moment'.

## 3.6 Trial Conditions

This trial was a 3-arm randomised control trial that compared 3 conditions in parallel including: a standardised intervention, a choice intervention and usual care control condition. Each trial condition is described in detail below.

#### *3.6.1.1 Standardised Intervention Condition*

The standardised (fixed-content) intervention used in this trial was previously developed and utilised by Ayling et al. (2019) in a pilot trial to assess whether it could effectively enhance the positive mood of older adults prior to influenza vaccination. Participants randomised to this intervention viewed a fixed-content video lasting 15-minutes, which was made up of high arousal stimuli in multiple forms including uplifting music (e.g., Elvis Presley), images (e.g., puppies, kittens, babies, flowers) and classic comedy (e.g., Michael McIntyre, Would I Lie to You). This intervention aimed to appeal to a range of different tastes for this age group, which resulted in a combination of these stimuli. The development of this intervention was with the collaboration of older adult public contributors (PPI groups) to advise on the most suitable content for this age group. This standardised intervention was viewed by participants on a computer tablet device with over-ear headphones.

#### *3.6.1.2 Choice Intervention Condition*

The second intervention arm was designed to examine whether individualising the content of the intervention would enhance its effects on positive mood. The individualisation of interventions can be referred to in various ways, such as tailored, personalised or choice, where these terms do not necessarily mean the same thing. Although considered to be individualisation, tailoring does not always mean an individual is given the option of choice. For example, a computer-tailored physical activity intervention requires participants to complete many questions and supply self-report data to receive feedback based on the information they have provided (Neville et al., 2009). Although this intervention is personalised to them and their responses to questions, there is no direct choice element for participants. Similarly, other tailored web-based interventions aiming to improve health outcomes across various medical conditions and patient populations follow the same suit (Lustria et al., 2013). Allowing participants to have an element of choice has been shown to lead to increased interest and motivation, where having an element of choice gives participants feelings of personal control (Cordova & Lepper, 1996). There are also psychological factors in play, whereby giving the option of choice can change behaviour, such as confidence or the belief that taking control of a situation will produce the desired outcome (McPherson & Britton, 2001). It is no surprise that people are receptive to having a choice because it has been ingrained in our society that restriction or elimination of choice makes people feel like they have lost an element of control that can lead to negative behaviours, such as aggression (Adelinis, 2005). Additionally, in the context of healthcare, there is evidence to suggest the importance of giving individuals choice when trying to achieve

behaviour change within healthcare. A study showed that when women are given a choice of two formats of a program to enhance heart disease management, the women in the choice intervention arm enhanced psychosocial and physical functioning up to one year compared with non- choice intervention comparators (Clark et al., 2008).

For these reasons, it was important to consider the implementation of choice when considering how to enhance the effects of the standardised intervention. Given the evidence suggesting the strength of choice interventions over standard interventions, a choice intervention was developed and incorporated as a condition for this trial. The development of this intervention was done in collaborating with industrial partner Rehab Studio LTD.

The key features of this choice intervention were designed to match that of the standardised intervention (e.g., length, comedy, music and imagery stimuli). Participants randomised to the choice intervention were able to select a total of 3 videos in succession (from a total of 12 available), each lasting roughly 5-minutes meaning the choice intervention was time-matched to the standardised intervention. The available categories to choose from included stand-up comedy (e.g., Tim Vines), situation-comedy (e.g., Would I Lie to You), music (e.g., Elvis Presley) and variety (e.g., Strictly Come Dancing, Britain's Got Talent). Participants viewed the intervention videos on a computer tablet device wearing over-ear headphones.

#### *3.6.1.3 Usual Care Control Condition*

Participants were instructed to sit in a study room for 15-minutes to replicate usual care. A countdown 15-minute timer was displayed on a tablet device screen to indicate the remaining time. All usual care participants were grouped in sessions together to ensure they did not have any second-hand intervention interaction, which assisted with replicating usual care. Participants were not prevented from talking to each other if they wished during this time.

## 3.7 Outcome Measures

### *3.7.1 Baseline Measures*

Baseline measures refer to those included within the baseline questionnaire (*Appendix E*) completed by participants following trial enrolment and prior to their first GP surgery visit. These outcomes included positive and negative trait affect, perceived stress and health status.

#### *3.7.1.1 Positive and Negative Trait Affect*

Positive and negative trait affect was measured at baseline using the Positive and Negative Affect Schedule Short Form (PANAS-SF) (Watson, Clark, & Tellegen, 1988). Participants were presented with 10 positive (e.g., excited) and 10 negative (e.g., ashamed) emotion adjectives, which they were asked to rate the extent to which they felt that way ‘‘in general” on a five-point scale (“very slightly or not at all” – “extremely)”. Positive and negative affect subscales were created by summing respective item scores, whereby higher scores denoted greater positive and negative trait affect. The PANAS-SF's internal reliability has previously been demonstrated to be very good, with consistent scores ranging from α = 0.86 to 0.90 for positive affect and α = 0.84 to 0.87 for negative affect (Cohen & Williamson, 1988) (where α = Cronbach's alpha). For this trial, the internal reliability for the PANAS-SF was excellent (positive affect scale was α = 0.89 and PANAS-SF negative affect scale was α = 0.87).

#### *3.7.1.2 Perceived Stress*

One of the most widely used psychological tools for measuring the perception of stress is the Perceived Stress Scale (PSS-10) (Cohen et al., 1983)Click or tap here to enter text.. This scale asked participants to rate recent experiences of stress over the previous month (e.g., “In the last month, how often have you felt that you were unable to control the important things in your life?”). Participants responded by selecting an option on a five-point scale (0= Never, 1= Almost Never, 2= Sometimes, 3= Fairly Often and 4= Very Often). As some items are reverse-coded, PSS scores were calculated by reversing relevant items before summing all 10-items on the scale together. A higher score denoted greater perceived stress. Previous literature has demonstrated good internal reliability (α > 0.74) for the PSS-10 (Barry et al., 2007). For this trial, the internal reliability was α = 0.88.

#### *3.7.1.3 Health Status*

Participant health status was assessed using the 12-item Short Form Health Survey (SF-12) (Ware et al., 1996, 1998). The SF-12 required participants to complete a series of questions about their health via a series of yes/no questions and Likert response questions, which assessed physical and emotional functioning (categories assessed include (1) physical and emotional functioning: limited a lot, limited a little, or not limited at all, (2) pain: excellent, very good, good, fair and poor, (3) mental health, vitality and social functioning: all of the time, most of the time, a good bit of the time, some of the time, a little of the time, and none of the time). SF-12 scores mental health and physical health scores were calculated in line with published guidelines using a weighted formula. Higher scores indicated greater mental and physical health respectively. The scoring output of the SF-12 produced a score for mental

health (mental competency score, MCS) and a separate score for physical health (physical competency score, PCS). Good internal reliability has been previously demonstrated using the SF-12, with α = 0.78 for PCS and α = 0.73 for PCS (Diener et al., 2009). For this trial, the internal reliability for MCS was α = 0.81 and PCS was α = 0.87.

### *3.7.2 State Affect Measures Collected on the Day of Vaccination*

Prior to, and immediately following intervention or usual care exposure, all participants completed the two Affective Slider Scales (Betella & Verschure, 2016), two visual analogue scales from the Dynamic Visual Analogue Mood Scales (Barrows & Thomas, 2017) and the Scale of Positive and Negative Experience (Diener et al., 2009). Of these measures, the Affective Slider happiness subscale was the primary outcome measures. This is because this was the scale that was used in the previous pilot study (Ayling et al., 2019) and determined the power calculations for this trial.

#### *3.7.2.1 Affective Slider*

The Affective Slider consisted of two visual analogue scales anchored with cartoon faces on each end to denote Happy/Sad and Alert/Sleepy (herein referred to as the Happiness and Alertness Affective Slider subscales) (*Appendix F*) (Betella & Verschure, 2016). Participants were asked to indicate "how you feel right now, that is, at the present moment" on these horizontal slider scales. A 100mm line allows for a 0 to 100 scoring system, where the division of the line into hundredths is sufficiently sensitive (Kontou et al., 2012). Higher scores for these slider scales indicated greater happiness or alertness.

#### *3.7.2.2 Dynamic Visual Analogue Mood Scales*

Very similar to the Affective Slider, the Dynamic Visual Analogue Mood Scales (DVAMS) consisted of the Happy/Sad and the Alert/Sleepy (herein referred to as the Happiness and Alertness subscales) (*Appendix F*) (Barrows & Thomas, 2016). The scales comprised of images of human faces, which can be displayed as male or female, whose expressions change when the marker's position on the vertical slider is moved up or down. The use of a 0-100 scale has been validated, whereby a higher score indicated a greater level of alertness or happiness. Very high internal validity of the DVAMS was shown with a Cronbach's α of 0.95 (Barrette et al., 2006).

#### *3.7.2.3 Scale of Positive and Negative Experience*

The Scale of Positive and Negative Experience (SPANE) consisted of 12-items, 6-items to assess positive feelings (e.g., pleasant) and 6-items to assess negative feelings (e.g., angry) (*Appendix F*) (Diener et al., 2009). Participants were asked to respond on a 5-point scale

("very slightly or not at all" – "extremely") to report the extent to which they felt a particular item "right now, that is, at the present moment". SPANE negative and SPANE positive subscale scores were calculated by summing positive and negative emotion items separately. Higher score denoted higher positive or negative affect, respectively. Good internal reliability for all scores have been shown with α = 0.87 and α = 0.81 for SPANE positive and SPANE negative, respectively (Diener et al., 2009). For this trial, SPANE positive ($\alpha$ = 0.89) and SPANE negative (α = 0.83) internal reliability was good.

### *3.7.3 Immune Measures*

Venous blood samples (8mL) were collected pre-vaccination and 4-weeks post-vaccination to measure influenza vaccine-specific antibody responses. On the day of collection, after being allowed to clot at room temperature, samples were centrifuged at 2000g for 10-minutes, after which sera were separated and stored at -80°C until analysis via Hemagglutination Inhibition (HAI) Assay and Enzyme-Linked Immunosorbent Assay (ELISA).

#### *3.7.3.1 HAI Assay to Measure Influenza- Specific Antibodies*

HAI assay is considered to be the gold standard for measuring antibody responses to vaccination and can be used to infer clinical protection based on pre-defined HAI titre thresholds. Conventionally, a post-vaccination titre of ≥40 (1:40) classifies individuals as seroprotected, or clinically protected, based on a 50% reduction in disease (Hobson et al., 1972). However, one of the biggest issues with this threshold in the context of influenza is that the likelihood of prior exposure to virus strains is very high, whether that be via previous vaccination or natural exposure (Beyer et al., 2004). This means that it is common to see individuals meeting this ≥40 HAI titre prior to vaccination, which makes it challenging to account for high pre- vaccination antibody levels. More recently, higher titre thresholds have been suggested, with a HAI titre of ≥160, based on a 90% reduction in disease, being more widely used as a metric of seroprotection to account for high pre-vaccination antibody levels (Hayney et al., 2014). For this reason, both thresholds will be investigated as part of the For-ME trial, where results are reported in *Chapter 5*.

##### 3.7.3.1.1 HAI Assay Protocol

Prior to the COVID-19 pandemic, I had planned to complete a 3-months secondment at Public Health England (PHE) to conduct the relevant HAI assay analyses on the collected pre- and post- vaccination blood samples. However, due to COVID-19, this secondment could not go ahead and instead these blood samples were analysed by PHE staff on our behalf. PHE have

provided an assay protocol, which is outlined below, that has been performed in line with the World Health Organisation guidelines (World Health Organisation, 2011).

In total, 1250 serum samples from 658 individuals (592 sample pairs and 66 unpaired samples) were stored in a -80°C freezer for analysis of three influenza strains, which were antigenically representative of those included in the 2019/20 trivalent inactivated influenza vaccine (aTIV): A/Brisbane/02/2018, A/Kansas/14/2017 and B/Colorado/06/2017.

The antigens for the three individual Haemagglutination Inhibition (HAI) analyses were egg grown (in-house) influenza viruses. The influenza B antigen (only) was extracted with Tween/Diethyl-Ether prior to analysis in order to increase assay sensitivity, which is standard procedure in the PHE laboratories for this virus subtype. All assays were performed using appropriate, pre-validated control sera (human and ferret origin) and titres of these controls were used to monitor and validate assay performance according to PHE standard operating procedure. All sera samples were treated with Receptor Destroying Enzyme from Vibrio cholerae (RDE II, Denka Seiken) prior to analysis. For analysis, pre-treated sera were examined in duplicate by serial dilution (two-fold; starting 1:10 and ending 1:1280) followed by incubation with antigen at a concentration of four Haemagglutination Units (HAU) each. Red blood cells (RBC) were used to detect inhibition of agglutination using turkey RBC for H1N1pdm09 and influenza B and guinea pig RBC for H3N2. Duplicate titres must agree with each other, which means they cannot vary more than 2-fold. When a greater than 2-fold difference was observed, analyses were repeated. This means that geometric coefficient of variation (GCV) to assess internal reliability of the assay was good. Samples with undetectable HAI titres ("negatives") were indicated as having tires of <10 were assigned a nominal value of 5. The highest possible titre within the dilution series of this assay was 1280, where by endpoint titrations (e.g., titres beyond a 1280-fold serum dilution were assigned a value of 1280) could not be conducted due to limitation of financial and staffing resources.

#### *3.7.3.2 ELISA to Measure IgG- Specific Antibodies*

ELISA analysis was carried out at The University of Nottingham. Antigens (A/Brisbane/02/2018 (H1N1); A/Kansas/14/2017 (H3N2) and B/Maryland/15/2016 (B) (referred to throughout this thesis as the B/Colorado/06/2017-like strain due to these strains being nearly antigenically identical) (National Institute of Biological Standards and Control)) were diluted to 0.5µgHA/mL with carbonate-bicarbonate buffer (Sigma-Aldrich), and 20µL was added into 384-well plates (NUNC MaxiSorp, Thermo Fisher Scientific). After overnight incubation at 4°C, plates were then blocked with 40µL of 3% Bovine Serum Albumin (BSA)

(Sigma-Aldrich) in phosphate buffered saline (PBS) with 0.05% sodium azide (Thermo Scientific) and incubated at 4°C for 3-5 days. Plates were aspirated and washed were in cycles of three, with 0.05% Tween-20 solution in PBS, between each step using a mechanical plate washer, with all incubations occurring at room temperature unless otherwise stated. Sera diluted at 1:2000 with 3% BSA in PBS and were then added to each antigen plate for 1-hour on a plate shaker and washed as described above. Following this, 20µL Horseradish peroxidase (HRP)- conjugated anti-human IgG (Sigma-Aldrich) diluted with PBS at 1:30,000 was added and incubated for 1-hour on a plate shaker and washed as described above. 20µL of Tetramethylbenzidine (TMB) (Thermo Scientific) was then added for 10-minutes before the reaction was stopped with 20µL of 2M sulphuric acid (at a 1:1 volume with the TMB substrate solution). Plates were then scanned at 450nm on a GloMax Explorer instrument (Promega).

Samples were analysed in duplicate, along with 16 influenza IgG-positive common sera sample controls, and BSA (Sigma-Aldrich) as a negative control. All dilutions and pipetting into plates were performed using a computerised pipetting system (Precision XS, BioTek) for accuracy. The 16 common sera samples on each plate were used to normalise results across plates and results are presented in terms of optical density values (ODs) with higher values indicating greater IgG antibody levels. Acceptable OD values aim to be between >1 to 3, whereby greater than 3 is usually considered to be out of the detection range for measuring absorbance at 450nm. For a full review of the optimisation and validation of this ELISA protocol refer to *Chapter 4*.

## 3.8 Data Management

Participant initials, date of birth, completed baseline questionnaire data, randomisation allocation and measured height and weight were all entered into an online data capture system (REDCap). To ensure the quality of the entered data for the baseline questionnaire booklets, a quality control measure known as continuous sampling was implemented (Gibson et al., 1994). This method requires one-time data entry into the online database, followed by a visual verification carried-out by a third party. Initially, the visual checks were done for the first 10 booklets. If no errors were detected in the first 10 booklets, the following checks were carried out on every 1 in 10 booklets. Due to the large volume of baseline questionnaires returned (*n*= 676), this method was deemed the most acceptable, as opposed to double data entry, which has also been indicated as an acceptable method of data entry checking for clinical trials (King & Lashley, 2000). However, evidence has suggested the gain of quality of double data entry may not justify the cost, in this case time, of the process

(Gibson et al., 1994). In the case of continuous sampling for this trial's data entry, when low error rates are observed, this method of data entry checking is much quicker than double data entry and does not compromise accuracy. By checking 1 in 10 booklets, an error rate of <7% is considered acceptable given the booklets' large quality.

## 3.9 Statistical Analysis

### *3.9.1 Primary Aim*

To address the primary aim of this trial, a series of one-way ANCOVA analyses were conducted to determine whether there were statistically significant differences between standardised, choice intervention and usual care groups, as well as determining which intervention type was superior. It is important to note that whilst several state affect outcomes were measured, the Affective Slider happiness subscale was the primary state affect outcome measures of this trial.

### *3.9.2 Secondary Aim*

One-way ANCOVAs (of ELISA data) and chi-squared analyses (of HAI data) were conducted to investigate whether group allocation predicted antibody responses to influenza vaccination, which was the secondary aim of this trial. Paired t test analyses were conducted to assess whether there were significant changes from pre- to post- vaccination antibody responses for each trial arm (standardised intervention, choice intervention and usual care groups).

### *3.9.3 Exploratory Analyses*

Exploratory analyses were carried out to address the following questions:

Question 1: To what extent did psychological factors at baseline influence pre- and post-intervention positive mood on the day of vaccination?

It was hypothesised that participants with high levels of perceived stress and negative trait affect measured at baseline would present with lower pre-intervention positive state affect scores. It was also hypothesised that higher levels of positive trait affect would be associated with higher levels of pre-intervention positive state affect. For participants with high levels of negative trait affect and perceived stress and/or low levels of positive trait affect measured at baseline, it was hypothesised that allocation to an intervention group would result in greater improvements in positive state affect due to there being larger room for improvement compared to participants with lower perceived stress and negative trait affect.

To address these questions, multiple regression analyses were conducted to investigate whether perceived stress (PSS scale) and trait mood (PANAS positive and PANAS negative) measured at baseline were significant independent predictors of pre-intervention state mood (SPANE positive, SPANE negative, affective slider happiness, affective slider alertness, DVAMS happiness and DVAMS alertness). Additionally, multiple regression analyses were conducted to assess whether psychological factors (PSS and trait affect) measured at baseline were predictors of post-intervention state affect to examine whether these factors were capable of influencing the effects of the intervention. Age and sex were taken into account as confounding factors.

Question 2: Did psychological factors measured at baseline predict antibody responses to influenza vaccination?

A plethora of psychological factors (perceived stress and trait affect) have been indicated to influence vaccination responses (refer to *Chapter 2*). It was therefore hypothesised that participants with high levels of stress and negative trait affect would present lower antibody responses to vaccination, and those with high positive trait affect would present higher 4-week post-vaccination antibody levels.

Multiple regression analyses were carried out to investigate whether 4-weeks post-vaccination ELISA OD levels could be predicted by perceived stress and trait affect. Binary logistic regression analyses were carried out to investigate whether perceived stress and trait affect could predict whether participants were classified as seroprotected for HAI thresholds ≥40 and ≥160. These analyses took into account confounding factors, age and sex, that have been indicated to influenza immune responses to vaccination.

In addition to these questions, a comparison between ELISA and HAI was conducted. Whilst these methods of measuring antibody responses to vaccination differ, it was hypothesised that they would be significantly correlated with each other. Analyses also investigated whether it was possible to determine ELISA OD values equivalent to HAI geometric mean values to determine whether the same proportion of participants were classified as seroprotected using these two different methods of measuring antibody responses to vaccination. These methods were compared by assessing their level of agreement to determine whether they could be interchangeable methods of measuring antibody responses to influenza vaccination.

Bivariate correlations (Pearson's) were computed to assess the relationship between the HAI geometric mean and ELISA OD values for each influenza strain of the vaccine. Bland-Altman analyses were conducted to determine whether ELISA OD levels and HAI geometric mean values have good agreement with each other (Bland & Altman, 1999).

All analyses presented reflect an intention-to-treat population. Analyses were performed using STATA software version 17.0 SE Standard Edition and SPSS software version 26. The results of these statistical analyses are reported in *Chapter 5*.

## 3.10 Chapter Summary

This chapter provides an overview of the For-ME trial design and methods. Trial procedures, conditions and outcome measures were described in detail, along with an outline of data management and statistical analyses. In the next chapter, the optimisation and validation of the ELISA protocol for assessing influenza- specific antibody levels from blood samples will be described (*Chapter 4*), followed by the For-ME trial analyses of results (*Chapter 5*).

# 4 Chapter 4: Enzyme-linked Immunosorbent Assay Optimisation and Validation to Measure Antibody Responses to Vaccination

## 4.1 Chapter Introduction

The gold standard for measuring antibody responses to influenza vaccination is the Hemagglutination Inhibition (HAI) assay, where conventionally a titre of ≥40 is used as the clinical protection (also referred to as seroprotection) threshold (Beyer et al., 2004; de Jong et al., 2003). However, the nature of the HAI assay means that there is no gradation between assay serial dilutions making it difficult to distinguish between samples (refer to *Chapter 3* for HAI protocol). Enzyme-Linked Immunosorbent Assay (ELISA) allows for the differentiation between samples due to this assay being a continuous outcome measure. For this reason, ELISA is considered to be a sensitive, flexible and low-cost laboratory technique readily used to measure antibody responses to vaccination (Coudeville et al., 2010; Lewnard & Cobey, 2018). For the analyses of For-ME trial blood samples, both HAI assay and ELISA were carried out to measure influenza-specific antibody responses to vaccination.

This chapter provides an overview of the optimisation and validation experiments conducted to optimise the measurement of influenza- specific antibody responses to vaccination by ELISA. Sections one and two of this chapter outline the initial ELISA protocol and validation experiments conducted, followed by section three and four that outlines the updated protocol and optimisation experiments that were subject to change due to COVID-19 impact (e.g., laboratory closure). The finalised ELISA protocol for the analyses of influenza- specific antibody responses to vaccination is described in detail here, and in *Chapter 3*.

## 4.2 Original Indirect Sandwich ELISA Analysis Protocol

This section outlines the "original" indirect sandwich ELISA analysis protocol utilised for validation and optimisation experiments conducted prior to the COVID-19 pandemic. Changes were made as a result of the COVID-19 pandemics impact on laboratory facility and resource access, which resulted in protocol changes (see S*ection 4.4* for the finalised ELISA protocol).

Serum preparation from whole blood was achieved by centrifuging up to 8mL of whole blood in a serum specific vacutainer tube (Beckon Dickinson) at 2000g for 10-minutes. Serum supernatant was then aliquoted into 2mL cryovials (Sigma Aldrich) to be frozen for ELISA analysis later.

#### *4.2.1.1 Standards, Controls and Equipment*

Standard curves were prepared using a Biotech Precision single-channel pipette liquid handling robot, in a 96 well plate (ThermoFisher Scientific). Each plate contained a calibrated human IgG (Sigma-Aldrich, I4506) standard curve (4µgHA/mL to 0.001µgHA/mL) in duplicate, as well as a negative control of PBS (Thermo Scientific) in duplicate. All washes, sera dilutions and addition of reagents were done using a Biotech Precision multichannel pipette liquid handling robot.

#### *4.2.1.2 Plate Coating with Antigen*

384 well plates (Merek) were separately coated with 20µL of the following 3 antigens, which were diluted to their working concentrations (0.5µgHA/mL) with 1µgHA/mL carbonate-bicarbonate buffer (Sigma-Aldrich): Influenza Antigen A/Brisbane/02/2018 (IVR-190) (H1N1) (NISBC, 18/238), Influenza Antigen A/Kansas/14/2017 (NYMC X-327) (H3N2) (NISBC, 19/104) and Influenza Antigen B/Maryland/15/2016 (also referred to as B/Colorado/06/2017- like strain) (NISBC, 18/100). Coated plates were incubated overnight at 4°C.

#### *4.2.1.3 Plate Washing*

After overnight incubation, plates were manually aspirated and washed with 0.05% Tween-20 in PBS (v/v) 3 times. This procedure was repeated after each reagent incubation, except for the final colorimetric substrate and stop solution stages of the assay.

#### *4.2.1.4 Blocking Non- Specific Antibody Binding*

The residual binding capacity of the plates were blocked by adding 40µL of 3% (v/v) BSA (Sigma-Aldrich) to each well. Plates were centrifuged at 2000g for 2-minutes and incubated on a shaker plate for 1-hour.

#### *4.2.1.5 Vaccine Specific Antibody Capture*

At this stage, all sera samples were thawed, mixed on a plate shaker, diluted to their working concentrations (1:2000) with PBS and were then added to each washed well. It is during this step that the influenza vaccine-specific antibodies bind to the antigens coated in each well. 20µL of each sample was plated in duplicate. Plates were centrifuged as described above and were incubated for 2-hours on a plate shaker.

#### *4.2.1.6 Detection Antibody*

After washing, 20µL biotinylated anti-human IgG (Vector Labs) diluted with PBS at 1:320,000 was added to each well. Plates were then centrifuged and incubated for 1-hour on a plate

shaker. At this stage, the detection antibody was left to bind to the previously captured IgG antibodies.

#### *4.2.1.7 Streptavidin- Enzyme Conjugate*

After washing, 20µL of enzyme conjugate streptavidin-HPR was added to each well, where the streptavidin of the enzyme complex bound to the biotinylated anti-human IgG. Plates were centrifuged and incubated in the dark on a plate shaker for 45-minutes.

#### *4.2.1.8 Colorimetric Substrate*

20µL of Tetramethylbenzidine (TMB) (Thermo Scientific) was added after washing, where the TMB substrate reacted with immobilised HRP conjugated to the biotinylated anti-human IgG to produce a blue solution. The colour change produced was proportional to the amount of biotin present, which meant a darker blue solution indicated a larger quantity of antigen-specific antibody present.

#### *4.2.1.9 Stop Reaction and Analyse*

After 15-minutes, without plate washing, this reaction was stopped using 20µL of 2M sulphuric acid ($H_2SO_4$) (at a 1:1 volume with the HRP/TMB substrate/enzyme solution), which had an immediate visual impact of turning the previously blue solution yellow. The absorbance was then measured on an ELISA reader (GloMax Explorer instrument (Promega) that output optical density (OD) values to determine antibody concentrations within each well. Plates were scanned at 450nM.

## 4.3 ELISA Optimisation and Validation Experiments (Part 1)

A series of experiments were conducted to optimise and validate the ELISA protocol (outlined in *Section 4.2*). The optimisation and validation experiments outlined in this section include: (Experiment 1) Checkerboard Assay, (Experiment 2) Reproducibility Testing 1 (Poor Standard Curve Fit), (Experiment 3) Reproducibility Testing 2 (Poor Sample Reliability) and (Experiment 4) Reproducibility Testing 3 (Poor H3N2 Reliability).

### *4.3.1 Experiment 1: Checkerboard Assay*

*Aim:* This experiment was conducted to determine the optimal ELISA plate coating antigen concentrations and sera sample dilutions. These two factors are essential to ensure that the obtained absorbance signals fall within the linear region of the 4-parameter logistic (4PL) sigmoidal IgG standard curves for all antigens. Symmetrical, 4PL sigmoidal standard curves are what we anticipate for immunoassay experiments, such as ELISA, as it best represents the kinetics of the antigen-antibody complex formation. For this experimental design, 4

randomly selected sera samples from the participants in the For-ME trial, 2 pre- and 2 post-vaccination samples, were tested against the following sera dilutions and antigen coating concentrations highlighted in *Table 4.1*.

**Table 4.1:** Checkerboard assay conditions consisting of five sera dilutions and four antigen coating concentrations tested for all 3 antigens.

| **Sera Dilutions** | **Antigen Coating Concentrations** |
|---|---|
| 1:1000 | 2µg/mL |
| 1:2000 | 1µg/mL |
| 1:4000 | 0.5µg/mL |
| 1:8000 | 0.25µg/mL |
| 1:16000 | - |

*Results:* When selecting the optimum coating antigen and sera concentrations, the obtained absorbance readings of desirable sera dilutions and antigen concentration combinations need to be compared with each corresponding antigen standard curves. As highlighted in *Figure 4.1,* the standard curve linear regions for all antigens were determined to be between an absorbance reading of 0.34-1.82, therefore corresponding to a human IgG concentration range of 0.017-0.5µg/mL. The observed differences between the curves were likely to be due to the timing differences between plate washing and reagent addition rather than differences relating to the antigen type. To assess the variation of standard curve duplicates on each plate, also known as intra-assay coefficients of variation (CV%) (ideal <5%), good intra-assay median CV%'s of <5% (IQR= <3%) were calculated for all standard curves. This therefore allowed for the determination of acceptable sera dilution and antigen concentrations illustrated *in Table 4.2, Table 4.3* and *Table 4.4* for H1N1, H3N2 and B antigens, respectively.

In addition to excellent median CV%'s for all standard curves, sample intra-assay reliability was shown to be excellent with median sample CV% values of 2.27% (IQR= 1.80%), 1.82% (IQR= 1.65%) and 2.00% (IQR=1.64%) for H1N1, H3N2 and B antigens respectively, where each sample was plated in triplet.

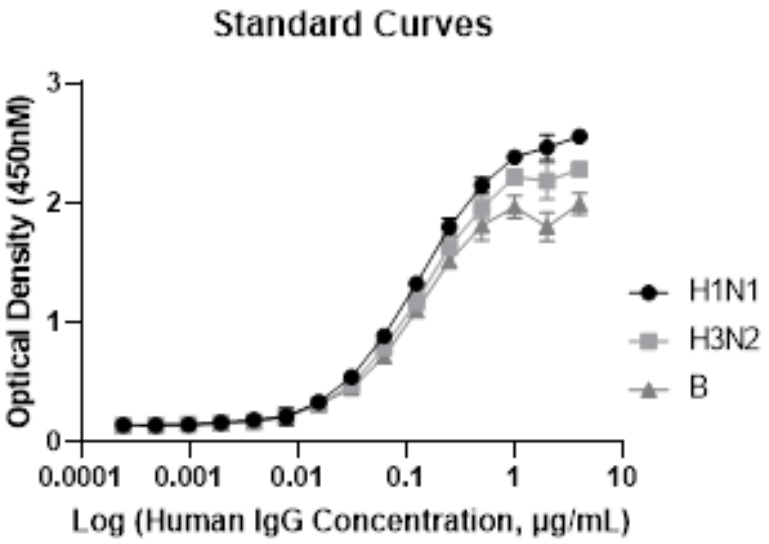



**Figure 4.1: Human IgG standard curves printed alongside H1N1, H3N2 and B antigen coated ELISA plates.** Standard curves for each plate were analysed in quadruplet, with an intra-assay CV's of <5% for all antigens. The identified linear regions for all three antigens are between an absorbance of 0.34-1.82, which corresponds to a human IgG concentration range of 0.017-0.5µg/mL.

Despite multiple combinations of sera dilution and antigen concentration being acceptable across all 3 antigens, selecting a combination that is nearer the middle of the standard curve linear region allows for flexibility if future analysed samples display much higher or much lower absorbance readings, and therefore human IgG concentrations. In the case of this checkerboard assay, serum dilutions of 1:2000 and 1:4000 were both deemed acceptable, however, 1:2000 was selected as the preferable dilution due to the absorbance ranges for all three antigens sitting more comfortably in the middle of the standard curve linear region of 0.34-1.82 (IgG concentration range of 0.017-0.5µg/mL). Although multiple antigen concentrations were also deemed acceptable, specifically 0.5µg/mL and 1µg/mL, the lowest acceptable concentration for all 3 antigens was selected for economic reasons due to the high cost of these antigens.

*Next step:* The findings of this checkerboard assay indicated the optimum serum dilution to be 1:2000 and the optimum antigen coating concentration to be 0.5µg/mL. The combination of these conditions were found to be the most optimal and were used for future experiments conducted below to test for reproducibility.

**Table 4.2**: Absorbance reading ranges for all 4 samples at varying sera dilutions and H1N1 antigen concentrations.

| | | Serum Dilution | | | | |
|---|---|---|---|---|---|---|
| | | **1:1000** | **1:2000** | **1:4000** | **1:8000** | **1:16000** |
| **Ag H1N1 Conc.** | **2µg/mL** | 1.12-2.08* | 0.78-1.89 | 0.51-1.59* | 0.29-1.19 | 0.24-0.81 |
| | **1µg/mL** | 0.99-2.00* | 0.71-1.80* | 0.47-1.48* | 0.32-1.12 | 0.24-0.76 |
| | **0.5µg/mL** | 0.91-1.90 | **0.64-1.55*** | 0.44-1.32* | 0.31-0.98 | 0.24-0.66 |
| | **0.25µg/mL** | 0.79-1.67 | 0.60-1.39 | 0.41-1.19* | 0.29-0.88 | 0.23-0.57 |

*Indicates sample absorbance ranges that fall within the linear region of the standard curve.

**Table 4.3**: Absorbance reading ranges for all 4 samples at varying sera dilutions and H3N2 antigen concentrations.

| | | Serum Dilution | | | | |
|---|---|---|---|---|---|---|
| | | **1:1000** | **1:2000** | **1:4000** | **1:8000** | **1:16000** |
| **Ag H3N2 Conc.** | **2µg/mL** | 1.36-1.83 | 1.01-1.63* | 0.66-1.27* | 0.45-0.92* | 0.30-0.60 |
| | **1µg/mL** | 1.12-1.77 | 0.87-1.54* | 0.59-1.20* | 0.39-0.83* | 0.26-0.57 |
| | **0.5µg/mL** | 1.01-1.65* | **0.70-1.39*** | 0.48-1.06* | 0.33-0.72 | 0.24-0.47 |
| | **0.25µg/mL** | 0.82-1.52* | 0.61-1.23* | 0.39-0.89* | 0.28-0.61 | 0.21-0.40 |

*Indicates sample absorbance ranges that fall within the linear region of the standard curve.

**Table 4.4**: Absorbance reading ranges for all 4 samples at varying sera dilutions and B antigen concentrations.

| | | Serum Dilution | | | | |
|---|---|---|---|---|---|---|
| | | **1:1000** | **1:2000** | **1:4000** | **1:8000** | **1:16000** |
| **Ag B Conc.** | **2µg/mL** | 0.97-1.65* | 0.69-1.42* | 0.45-1.09* | 0.31-0.77 | 0.24-0.51 |
| | **1µg/mL** | 0.92-1.61* | 0.64-1.37* | 0.45-1.04* | 0.31-0.75 | 0.24-0.49 |
| | **0.5µg/mL** | 0.89-1.50* | **0.60-1.25*** | 0.41-0.97* | 0.30-0.68 | 0.22-0.45 |
| | **0.25µg/mL** | 0.78-1.32* | 0.55-1.08* | 0.38-0.86* | 0.28-0.58 | 0.22-0.40 |

*Indicates sample absorbance ranges that fall within the linear region of the standard curve.

### *4.3.2 Experiment 2: Reproducibility Testing 1 (Poor Standard Curve Fit)*

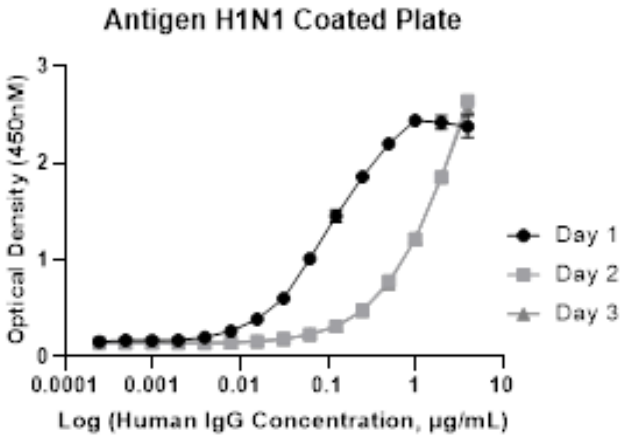
*Aim:* To assess the reproducibility of the ELISA assay, 175 randomly selected human sera samples (including pre-and post-vaccination blood samples) were processed three times, each on separate days within the same week. Following the procedure outlined in *Section 4.2)*, sera samples were tested, and coefficients of variation (CV%) were calculated to assess both intra-assay reliability and inter-assay reliability for standard curves and samples.

*Results:* Intra-assay reliability was good for all human IgG standard curves, with median CV%'s of <6% (IQR= <5%) across all 3 testing days. However, poor standard curve inter-assay reliability was observed with calculated median CV%'s of 36.27% (IQR= 59.52%) (H1N1), 28.45% (IQR= 59.15%) (H3N2) and 24.00% (IQR= 51.58%) (B), where conventionally we would aim for CV% values of less than 20% (Reed et al., 2002). Large IQR values also indicated a large amount of variability between the standard curves on each day.

In addition to this, we would conventionally aim for a symmetrical, S-shaped standard curve to sufficiently interpolate and analyse data. However, for the first round of reproducibility testing, each antigen standard curve, displayed in *Figure 4.2*, indicated poor goodness of fit for 2 of the 3 testing days. Day 1 presented with a standard curve that aligns with what we would have expected to see, which means the differences observed lie within potential differences in the protocol for days 2 and 3. The likely difference was speculated to have been due to the human IgG working concentration of 4µg/mL not being freshly prepared each day, and was instead only freshly prepared on day 1 and used over the 3 experimental days. It is possible that storing this antibody overnight in an undesirably low concentration before use had the potential to alter its binding ability due to the occurrence of antibody degradation and change in performance.

To assess inter-assay reliability, sample antigen concentrations needed to be interpolated from the standard curve of each plate. This is because we are likely to see OD variation of the same standard curves day-to-day due to alterations of laboratory conditions, such as humidity and temperature. This highlights the value of using a standard curve to standardise across experiments conducted on different days. As mentioned previously, this experiment produced poor standard curves, which means sample interpolation was not possible and subsequently sample inter-assay reliability could not be determined. However, sample intra-assay reliability could be determined using OD values, where excellent median CV%'s of <4% (IQR= <4%) were calculated for all sample antigens across the 3 experimental days.

*Next step:* Poor standard curves were produced in this round of reproducibility testing. The next section aimed to test whether human IgG storage played a role in the production of the poor standard curves produced within this section of reproducibility testing.



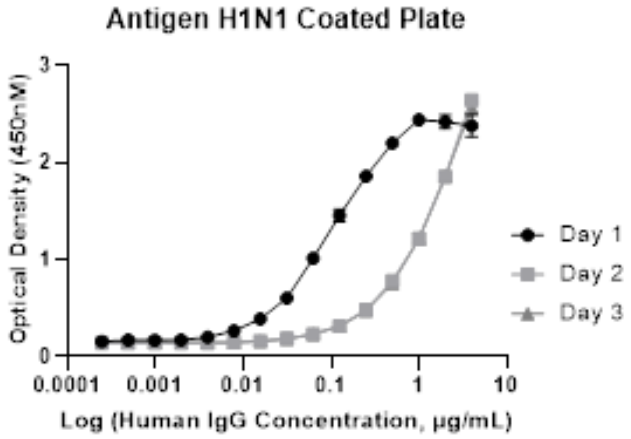




**Figure 4.2: Absorbance signals for standard curves (4µg/mL to 0.001µg/mL), on 3 consecutive days, for ELISA plates separately coated with antigens H1N1, H3N2, B.** For all 3 antigens, days 2 and 3 display poor goodness of fit standard curve, compared to day 1 that displays a good fit standard curve.

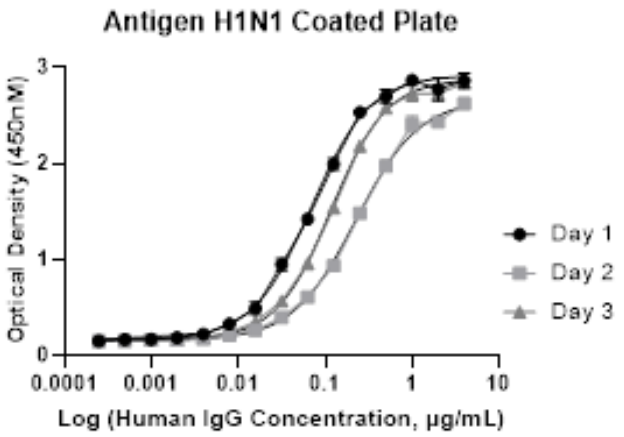
### *4.3.3 Experiment 3: Reproducibility Testing 2 (Poor Sample Reliability)*

*Aim:* For this set of reproducibility analyses, a new set of 175 pre- and post-vaccination samples were thawed and used for analyses, to eliminate the chance of biological degradation that may have occurred over a week, where the previously thawed samples were stored in the fridge. Following the previous experiment, this round of testing aimed to repeat the above experiment, however, this time using a fresh aliquot of 4µg/mL human IgG prepared at the start of each day to avoid the chance of biological degradation of the antibody, to achieve desirable standard curves on all testing days.

*Results:* The preparation of new human IgG each day proved successful, as demonstrated in *Figure 4.3*, where all three days produced desirable, symmetrical sigmoidal standard curves. Standard curve intra-assay reliability was satisfactory, with median CV%'s of <9% (IQR= <10%) for all antigens across all 3 experimental days, which is slightly greater than the ideal CV% of <5%. Inter-assay standard curve reliability was good, with median CV%'s of 19.84% (IQR= 23.45%) (H1N1), 17.13% (IQR= 23.71%) (H3N3) and 17.32% (IQR= 19.56%) (B) (ideal CV% <20%). Sample consistency was highlighted with good intra-assay reliability indicated by CV% values of <4% (IQR= <5%) for all sample antigens across the 3 days.

Despite an improvement in the shape of the standard curves compared to reproducibility testing 1 (*Figure 4.2*), as well as good sample intra-assay reliability, sample inter-assay reliability was extremely poor with median CV% values of 36.00% (IQR= 17.90%), 56.51% (IQR= 16.58%) and 22.00% (IQR= 12.62%) for H1N1, H3N2 and B antigens, respectively. Conjectures about these poor sample reliability figures suggested the possible issue was due to the plates completely drying between each addition of reagent. When using the liquid handling robot, each plate remained dry for at least 3-minutes, and often longer than this, which allowed for complete plate drying. Wells that are completely dry can lead to the inactivation of plate components, resulting in a negative impact on assay performance, such as poor reproducibility.

*Next step:* Liquid handling robot adjustments were made to ensure each plate could be manually aspirated directly before the addition of reagent, therefore minimising the timeframe in which plates remain dry. This was explored in the third set of reproducibility testing outlined in the next section of this chapter.



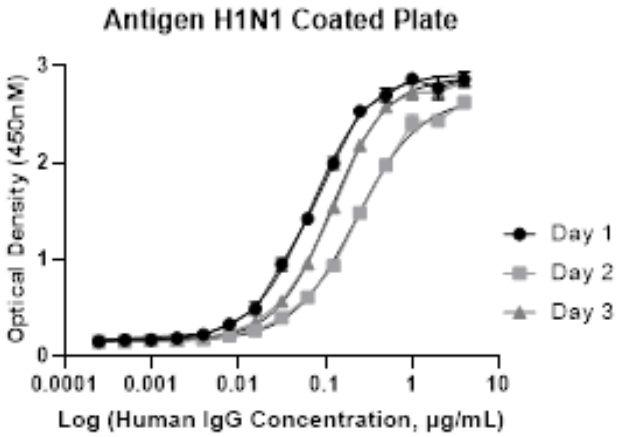




**Figure 4.3: Absorbance signals for standard curves (4µg/mL to 0.001µg/mL), on 3 consecutive days, for ELISA plates separately coated with antigens H1N1, H3N2, B.** All standard curves display desirable, goodness of fit curves

### *4.3.4 Experiment 4: Reproducibility Testing 3 (Poor H3N2 Reliability)*

*Aim:* This stage of reproducibility testing sought to resolve the issue of poor sample reproducibility by reducing the time each ELISA plate was left to dry.

*Results:* Consistent with the previous experiment, standard curve intra-assay median CV% values were calculated at <7% (IQR= <6%), which were considered to be satisfactory. Also, good intra-assay sample reliability was indicated with median CV% values of <4% (IQR= <4%).

The reduced dry-time of the ELISA plates resulted in a drastic increase in both inter-assay sample reliability and inter-assay standard curve reliability. Compared to the previous reproducibility testing experiment, standard curve inter-assay reliability for plates coated with H1N1, H3N2 and B antigens drastically increased from median CV% values of 19.84% (IQR= 23.45%), 17.13% (IQR= 23.71%) and 17.32% (IQR= 19.56%) to 5.85% (IQR= 3.59%), 7.96% (IQR= 2.79%) and 4.31% (IQR= 5.72%).

In addition to this, there was a considerable improvement in inter-assay sample reliability, with median CV% values of 10.95% (IQR= 10.37%) (H1N1), 20.57% (IQR= 10.55%) (H3N2) and 10.33% (IQR= 5.44%) (B) were also produced. The inter-assay CV% value for antigen H3N2 is slightly elevated, compared to the other two antigens, and is very much at the higher end of the ideal CV% value of 20% we would conventionally aim for. A possible explanation for this could be that the absolute concentration values are very low, as low as 0.085µg/mL for some samples for antigen H3N2, and is therefore likely to lead to greater variation of highly sensitive CV% calculations.

*Next step:* The positive outcome of this experiment led to the stipulation of processing each antigen plate in succession, through each stage of the ELISA experimental procedure, to avoid the plates drying by only aspirating plates directly before addition of reagent. However, the inter-assay median CV% value for antigen H3N2 was still higher than desired and suggested a standard curve alternative needed to be considered. This may be because the standard curve is serially diluted IgG in PBS, which is not antigen-specific and therefore might be a poor true concentration reference to use. Despite the mostly positive outcome of this reproducibility experiment, additional experiment alterations needed to be made including: blocking incubation time, detection antibody type and a standard curve alternative (pooled sera sample controls and common sera sample controls). These are all described in more detail in the next section.

## 4.4 Final Direct Sandwich ELISA Analysis Protocol

### *4.4.1 Impact of the COVID-19 Pandemic*

At this point in the reproducibility testing process, the COVID-19 pandemic emerged. Laboratory work was halted for several months due to the COVID-19 national lockdown resulting in laboratory closure. After several months, laboratory opening was granted on the basis of increased health and safety precautions. These included the wearing of surgical masks and visors at all times and a maximum laboratory space capacity of 6 people, which was monitored via the use of an online booking system. Reduced laboratory capacity, the online booking system, and increased personnel needing to use the liquid handling robots for personal and COVID-19 research purposes meant that some adaptations needed to be made to the previously outlined ELISA protocol.

### *4.4.2 Updated ELISA Protocol*

The protocol changes were made to increase adaptability and flexibility of the experimental protocol and included the following: (1) plate blocking incubation time was changed to 3-5 days from 1-hour, (2) a new detection antibody type was used to eliminate the addition of enzyme conjugate streptavidin-HPR, therefore changing the protocol from indirect to direct ELISA and (3) common control samples were used instead of a standard curve as part of the experimental controls. The final direct sandwich ELISA protocol used for reproducibility testing experiments 4 and 5, as well as For-ME pre- and post- vaccination blood sample analyses, is described below (essential changes made from the previous protocol are highlighted in **BOLD**).

#### *4.4.2.1 Controls and Equipment*

Sera dilutions and addition of reagents were done using a computerised multichannel pipette liquid handling robot (Precision XS, BioTek). Each plate contained a series of common samples in duplicate, as well as a negative control of BSA (Sigma-Aldrich) in duplicate.

#### *4.4.2.2 Plate Coating with Antigen*

384 well plates (NUNC MaxiSorp, Thermo Fisher Scientific) were separately coated with 20µL of the following 3 antigens, which have been diluted to their working concentrations (0.5µgHA/mL) with 1µgHA/mL carbonate bicarbonate buffer (Sigma-Aldrich): Influenza Antigen A/Brisbane/02/2018 (IVR-190) (H1N1) (National Institute of Biological Standards and Control (NIBSC), 18/238), Influenza Antigen A/Kansas/14/2017 (NYMC X-327) (H3N2) (NIBSC, 19/104) and Influenza Antigen B/Maryland/15/2016 (referred to as B/Colorado/06/2017-like strain) (NIBSC, 18/100). Coated plates were incubated overnight at 4°C.

#### *4.4.2.3 Plate Washing*

Plates were aspirated and washed in cycles of three, with 0.05% Tween-20 solution in PBS, between each step using a **mechanical plate washer**.

#### *4.4.2.4 Blocking Non- Specific Antibody Binding*

The residual binding capacity of the plates was blocked by adding 40µL of 3% (v/v) BSA (Sigma-Aldrich) **with 0.05% sodium azide** (Thermo Scientific) to each well. Addition of sodium azide was used as a preservative to inhibit contaminants' growth, such as bacteria and fungi. Plates were centrifuged at 2000g for 2-minutes **and incubated at 4°C for 3-5 days**.

#### *4.4.2.5 Vaccine Specific Antibody Capture*

Serum preparation from whole blood was achieved by centrifuging up to 8mL of whole blood in a serum specific vacutainer tube (Beckon Dickinson) at 2000g for 10-minutes. After washing as described above, serum supernatant was then aliquoted into 2mL cryovials (Sigma-Aldrich) to be frozen for ELISA analyses later. At this stage, all sera samples were thawed, mixed on a plate shaker, diluted to their working concentrations with **3% (v/v) BSA** (Sigma-Aldrich) and were then added to each washed well. It is during this step that vaccine-specific antibodies bound to the antigens coated in each well. 20µL of each sample was plated in duplicate. Plates were centrifuged as described above and were incubated for 1-hour on a plate shaker.

#### *4.4.2.6 Detection Antibody*

After washing as described above, 20µL **HRP- conjugated anti-human IgG (Sigma-Aldrich) diluted with PBS at 1:30,000** was added to each well. Plates were then centrifuged and incubated for 1-hour on a plate shaker. At this stage, the detection antibody was left to bind to the previously captured IgG antibodies.

#### *4.4.2.7 Colorimetric Substrate*

20µL of Tetramethylbenzidine (TMB) (Thermo Scientific) was added after washing, where the TMB substrate reacted with immobilised peroxidase-conjugated to the biotinylated anti-human IgG, to produce a blue solution. The colour change produced is proportional to the amount of biotin present, which means a darker blue solution indicates a larger quantity of antigen-specific antibody present.

#### *4.4.2.8 Stop Reaction and Analyse*

After **10-minutes**, without plate washing, this reaction was stopped using 20µL of 2M sulphuric acid ($H_2SO_4$) (at a 1:1 volume with the HRP/TMB substrate/enzyme solution), which

had an immediate visual impact of turning the previously blue solution yellow. The absorbance was then measured on an ELISA reader (GloMax Explorer instrument (Promega), which output optical density (OD) values to determine antibody concentrations within each well. Plates were scanned at 450nM.

## 4.5 ELISA Optimisation and Validation Experiments (Part 2)

### *4.5.1 Experiment 5: Reproducibility Testing 4 (New Detection Antibody, Pooled Control Samples, Blocking Incubation Time)*

*Aim:* Following the need for protocol alterations after the completion of *Experiment 4*, this experiment aimed to investigate 3 essential changes. Firstly, this experiment tested the impact of blocking incubation time on reproducibility, by testing 3-, 4- and 5-day incubation periods. Secondly, both the feasibility and concentration of a new detection antibody was tested, whereby a working concentration of 1:30,000 was used. Finally, the poor standard curve reproducibility seen for all antigens indicated the need to explore the use of an alternative. In this experiment, 30 sera samples, which were positive for all antigens, were combined and diluted to high (1:500), medium (1:4000) and low (1:16,000) working concentrations. Consistent with the previous reliability experiments, all selected human sera samples (*n*= 194) were processed three times, each on separate days within the same week to test the alterations mentioned above.

*Results:* Intra-assay reliability of the pooled sera samples, for all antigens, was good for day 1, with a median CV% value of <4% (IQR= <5%), however extremely poor intra-assay reliability for days 2 and 3, with median CV% values of <8% (IQR= <22%) and <13% (IQR= <12%) respectively. Additionally, pooled sera sample inter-assay reliability was variable across the antigens with median CV% values of 12.80% (IQR= 7.0%) (H1N1), 8.37% (IQR= 8.74% (H3N2) and 20.90% (IQR= 9.56%) (B), whereby Antigen B in particular was poor. The lowest pooled sera concentration of 1:16,000 had extremely low OD readings for all antigens (OD range of 0.227-0.391), which is only slightly above background (OD value of 0.200). As a result, these extremely low OD readings could be the reason behind inconsistent pooled sera sample reliability.

Sample intra-assay reliability was good for all sample antigens across the 3 experimental days, with median CV% values of <5% (IQR= <10%). However, sample inter-assay reliability was variable with median CV% values of 15.06% (IQR= 13.69%), 13.36% (IQR= 6.91%) and 25.28% (IQR= 9.46%) for H1N1, H3N2 and B antigens, respectively. Consistent with the pooled sample reliability observations, antigen B was very poor.

*Next step:* Due to the inconsistent pooled sera sample reliability observed in this experiment, the next step was to test the use of common sera samples as the experimental controls, as an alternative to pooled sera samples.

### *4.5.2 Experiment 6: Reproducibility Testing 5 (Common Sample Controls)*

*Aim:* This experiment investigated the use of 16 common sera sample controls to determine whether regression analyses could be used to normalise results across plates to improve inter-assay reliability, particularly for antigen H3N2 which had been performing poorly. The use of common sera samples meant that the same sera samples were used as control samples on all antigen plates, across all 3 experimental days. The blocking incubation periods (3-, 4- and 5- days) and the new detection antibody concentration (1:30,000) were used in the same manner as described in *Experiment 5*.

*Results:* Excellent sample intra-assay reliably was consistent with previous experiments, whereby median CV% values across all 3 testing days were <5% (IQR= <5%) for H1N1, <3% (IQR= <4%) for H3N1 and <5% (IQR= <6%) for B.

Contrary to the previously outlined reproducibility experiments, this experiment did not rely on using a standard curve for interpolation to ensure day-to-day variations of OD values produced reliable results. However, the use of a standard curve has proved challenging, and so has the use of pooled sera sample controls. In this experiment, 16 common sera samples on each plate were used to normalise results across plates and results were presented in terms of OD values, with higher values indicating greater IgG antibody levels. Performed regression analyses estimated adjusted sera sample OD and CV% values. Regression analyses was conducted to produce regression models to both include and exclude a constant, whereby both models resulted in a drastic improvement in inter-assay sample reliability. The adjusted inter-assay sample median CV% values, which followed standard regression analyses protocol and therefore included a constant within the regression model, were excellent with values of 10.17% (IQR= 11.97%) (H1N1), 9.03% (IQR= 8.23%) (H3N2) and 7.72% (IQR= 6.33%) (B). Furthermore, these CV% values were further improved when a constant was excluded as part of the regression model equation, resulting in inter-assay median CV% values of 8.90% (IQR= 10.26%) (H1N1), 8.22% (IQR= 6.29%) (H3N2) and 6.72% (IQR= 4.96%) (B).

*Next Step:* Excellent regression adjusted median CV% values, and therefore excellent experiment reliability and reproducibility, concluded the series of ELISA optimisation and validation experiments. Also, excellent sample reliability suggested the plate blocking

incubation periods (3-, 4- and 5-days) and the use of new detection antibody concentration (1:30,000) were both acceptable. As a result, no further modifications were made to the ELISA protocol. Blood samples collected as post of the For-ME trial were analysed following this protocol (*n*=656 pre-vaccination and *n*=592 post-vaccination blood samples). The statistical analyses and results are outlined and explained in detail in *Chapter 5*.

## 4.6 Chapter Summary

This chapter has described the experiments conducted to optimise and validate the ELISA assay utilised to measure influenza-specific antibody responses to vaccination as part of the For-ME trial. Firstly, the initial indirect ELISA protocol was outlined along with a series of reproducibility experiments. The impact of COVID-19 on facility and resource access resulted in ELISA protocol changes, whereby all changes to this protocol are clearly highlighted. Final reproducibility experiments were conducted to optimise and validate the altered ELISA protocol. This final protocol was deemed sufficient for For-ME trial blood sample analyses. Pre- and post- vaccination blood samples were analysed, with statistical analysis and results explained in detail in *Chapter 5*.

# 5 Chapter 5: For-ME Trial Results: Enhancing Positive Mood and Assessing Influenza Vaccination Responses

## 5.1 Chapter Introduction

This chapter presents the results from the For-ME trial (For-ME trial design and methods are outlined in *Chapter 3*). The start of this chapter will introduce information about the trial participants, whereby participant demographics will by outlined. Baseline health, stress and mood measure (PSS, PANAS-SF, SF-12) characteristics will also be reported for the whole cohort and for both intervention groups and the control group, along with the pre- and post-intervention mood measures (Affective Slider, DVAMS, SPANE) and pre- and post-vaccination antibody measures (HAI, ELISA).

Once the For-ME data has been described, analyses relating to the following aims will be presented and discussed:

- Primary aim: To explore whether a standardised and/or choice intervention can enhance positive mood (measured by the Affective Slider Happiness subscale) compared to usual care in older adults, and to identify which intervention type was superior.
- Secondary aim: To explore whether positive mood, on the day of influenza vaccination, predicted antibody responses (measured by ELISA and HAI) to influenza vaccination in older adults.

In addition to these aims, exploratory analyses will also be presented for the following:

- To explore whether baseline psychological factors (perceived stress and trait mood) were associated with mood on the day (pre- and post-intervention mood) of influenza vaccination.
- To explore whether baseline psychological factors (perceived stress and trait mood) were associated antibody responses to influenza vaccination.
- To compare two different methods of measuring antibody responses to vaccination (ELISA versus HAI).

The end of this chapter will then outline the key outcomes for the For-ME trial results.

## 5.2 Treatment of Data

### *5.2.1 Testing Assumptions*

Normality of distribution of all variables was assessed via the calculation of skewness and the visual inspection of histograms prior to performing parametric analyses. When non-normality was observed in histograms, skewness statistics outside of a range of -2 to +2 were considered to be sufficiently skewed to require transformation (George & Mallery, 2010). Pre- and post-intervention SPANE negative affect subscale scores were significantly positively skewed. Transformation attempts (log (natural, 2 and 10), square root and inverse) of these variables were all unsuccessful. Therefore, in subsequent analyses the original scores were used, with non-parametric tests conducted where possible. Findings related to this variable should therefore be treated with appropriate caution. ELISA optical density (OD) variables (pre- and post-vaccination for all three vaccination strains) were significantly positively skewed. Untransformed OD values are reported in the descriptive statistics, however transformed (log2) variables were utilised for any analyses. Univariate outliers were assessed either via the visual inspection of box plot graphs and removed, if necessary, prior to analyses.

Homogeneity of variance was assessed for group comparisons (e.g., ANCOVA) using Levene's test, with significant values ($p$<0.05) indicating unequal variances. Test statistics were reported where equal variances were not assumed, in this case for the following: post-intervention happiness Affective Slider scale score, pre- and post-intervention alertness Affective Slider scale scores, pre- and post-intervention SPANE negative affect subscale scores, post-intervention happiness and alertness DVAMS scale scores, ELISA post-vaccination B antigen optical density, ELISA pre-vaccination H1N1 antigen optical density. Despite these variances, the large data set size and nature of parametric testing suggest that ANCOVA is a robust method of analysis that can still be carried out for group comparisons.

Chi-squared assumptions were met by ensuring all variables were categorical and independent of each other. Cells in the contingency table were mutually exclusive and expected values of calls were equal to or greater than 5 for 80% of cells.

For regression models, multicollinearity was assessed by bivariate Pearson's correlations and variation inflation statistics. Correlations greater than 0.8 and variance inflation factors above 10 were considered to be unacceptable levels of multicollinearity. Variables with significant correlations, and correlations of 0.8 or greater, were not used within the same regression models and instead separate regression model analyses were conducted.

### *5.2.2 Statistical Approach*

*Primary aim:* To address the primary aim of this trial, a series of one-way ANCOVA analyses were conducted to determine whether there were statistically significant differences between standardised and choice intervention and usual care groups, and to identify which intervention type was superior, in terms of post- intervention state affect (SPANE positive, SPANE negative, Affective Slider happiness, Affective Slider alertness, DVAMS happiness and DVAMS alertness). It is important to note that whilst several state affect outcomes were measured, the Affective Slider happiness scale was the primary state affect outcome measure of this trial, with pre-intervention scores used as the co-variates. Paired t test analyses were carried out to assess whether there were significant changes in state affect scores from pre- to post-intervention.

*Secondary aim:* One-way ANCOVAs (of ELISA data) and chi-squared analyses (of HAI data) were done to investigate whether group allocation predicted antibody responses to influenza vaccination, which was the secondary aim of this trial. Paired t test analyses (of ELISA data) and McNamar analyses (of HAI data) were conducted to assess whether there were significant changes from pre- to post-vaccination antibody responses for each trial arm (standardised intervention, choice intervention and usual care groups).

Exploratory analyses were carried out to answer the following questions:

(1) To what extent did psychological factors at baseline influence pre-intervention positive mood on the day of vaccination? Multiple regression analyses were conducted to investigate whether perceived stress (PSS scale) and trait mood (PANAS-SF positive and PANAS-SF negative) measured at baseline were significant independent predictors of pre- and post-intervention state affect (SPANE positive, SPANE negative, Affective Slider happiness, Affective Slider alertness, DVAMS happiness and DVAMS alertness). Age and sex were taken into account as confounding factors.

(2) Can psychological factors measured at baseline predict antibody responses to influenza vaccination? Multiple regression analyses were carried out to investigate whether 4-weeks post- vaccination ELISA OD levels can be predicted by perceived stress and trait affect measured at baseline. Binary logistic regression analyses were carried out to investigate whether perceived stress and trait affect measured at baseline can predict whether participants were classified as seroprotected for HAI thresholds ≥40 and ≥160. These analyses took into account confounding factors, age and sex, that have previously been indicated to influence immune responses to vaccination.

(3) Were the two different methods of measuring antibody responses to vaccination, HAI and ELISA, highly correlated and did they have good agreement? Pearson's correlation analyses were carried out for each influenza strain to assess the strength of the association between these methods. Bland-Altman analyses (Bland & Altman, 1999) were conducted to determine whether these methods were in good agreement and to examine equivalence.

Statistical analyses were presented to reflect an intention-to-treat population. Analyses were performed using STATA software version 17.0 SE Standard Edition and SPSS software version 26.

## 5.3 Descriptive Statistics

### *5.3.1 Trial Participants*

A total of 13 primary care GP surgeries within the East Midlands area were recruited for this trial, from which a total of 7580 eligible patients were invited to take part. Positive responses of expressing an interest in taking part was received by 848 patients, which was an uptake of 11.2% (slightly greater than the 10% assumed based on previous studies). Of these initial expressions of interest, a total of 705 ultimately agreed to participate and were randomised to a trial arm, with 654 attending the first GP surgery visit and providing written consent to take part. A summary of the flow of participants throughout the trial, including reasons for loss to follow-up, are highlighted in *Figure 5.1*. Participant dropout between randomisation and consent (*n*= 51, 7.2%) occurred due to randomisation taking place at the time of participant recruitment, often days or weeks prior to the first GP surgery visit. It was important to randomise participants at this point to ensure intervention and usual care sessions were booked separately to avoid contamination concerns observed in the previous pilot trial (Ayling et al., 2019). Attrition was low, with 617 participants of the 654 who fully consented (94.3%) attending the 4-week follow-up GP surgery visit for post-vaccination blood sample collection.

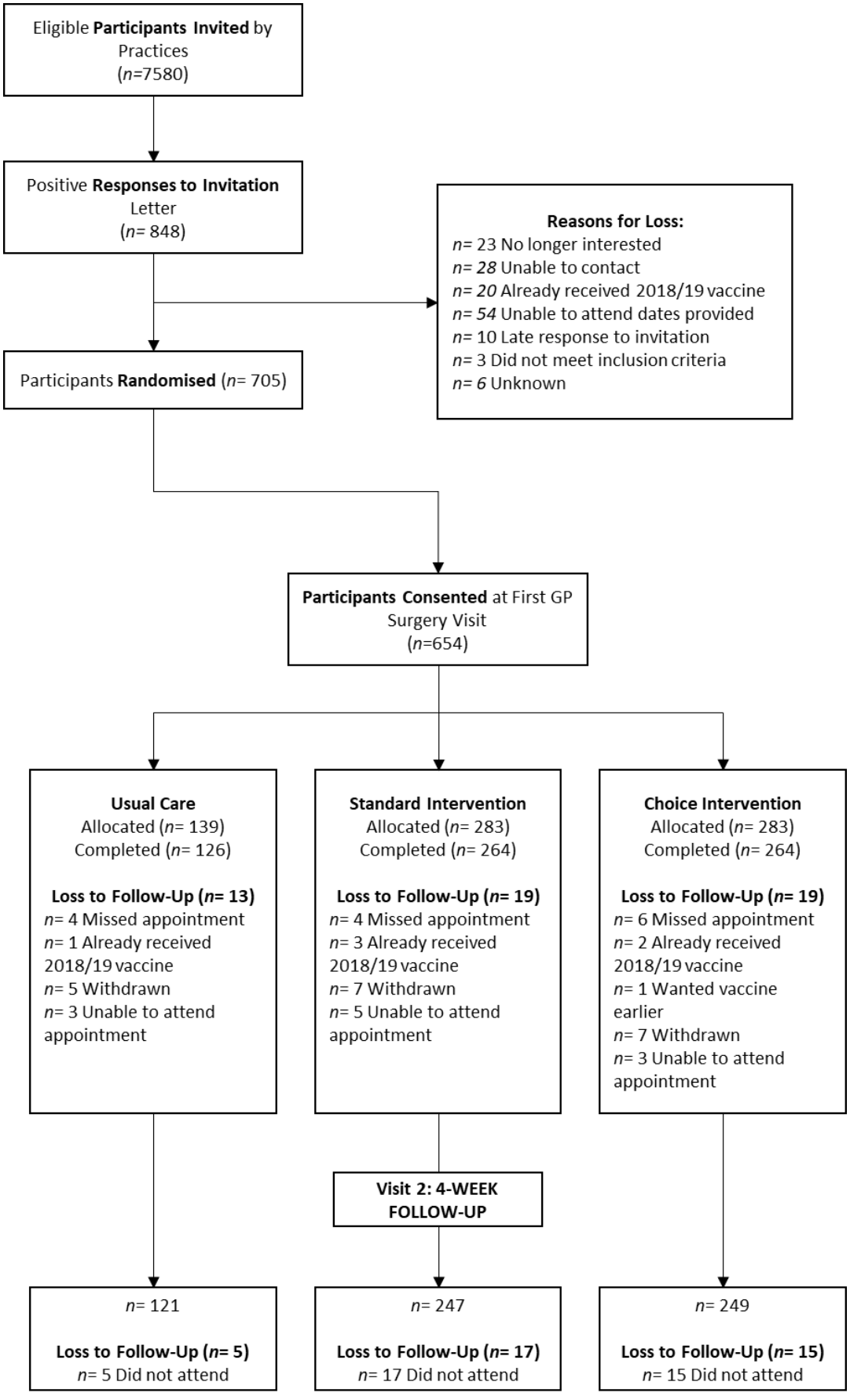



**Figure 5.1:** Flow diagram of participants in For-ME trial.

### *5.3.2 Participant Demographics*

*Figure 5.2* highlights (in red) the baseline measures assessed pre-vaccination as part of the For-ME trial. At T0, a baseline questionnaire assessed the following: demographic characteristics, trait affect (PANAS-SF) (Watson, Clark, & Carey, 1988), perceived stress (E. H. Lee, 2012) and health status (Ware et al., 1998). Within this section, cohort and trial arm descriptive statistics for these measures are presented.

Baseline demographic information of the 654 older adult participants is presented in *Table 5.1*. Females accounted for just over half of the trial participants (53.7%). This cohort was predominantly white (96.3%), married (64.5%) and lived independently (93.4%). School was the most commonly reported level of education (47.9%) and participants had an average body mass index (BMI) of 27.7, which is classified as moderately overweight. The average age of the cohort was just under 73 years (mean= 72.89, SD= 5.07).

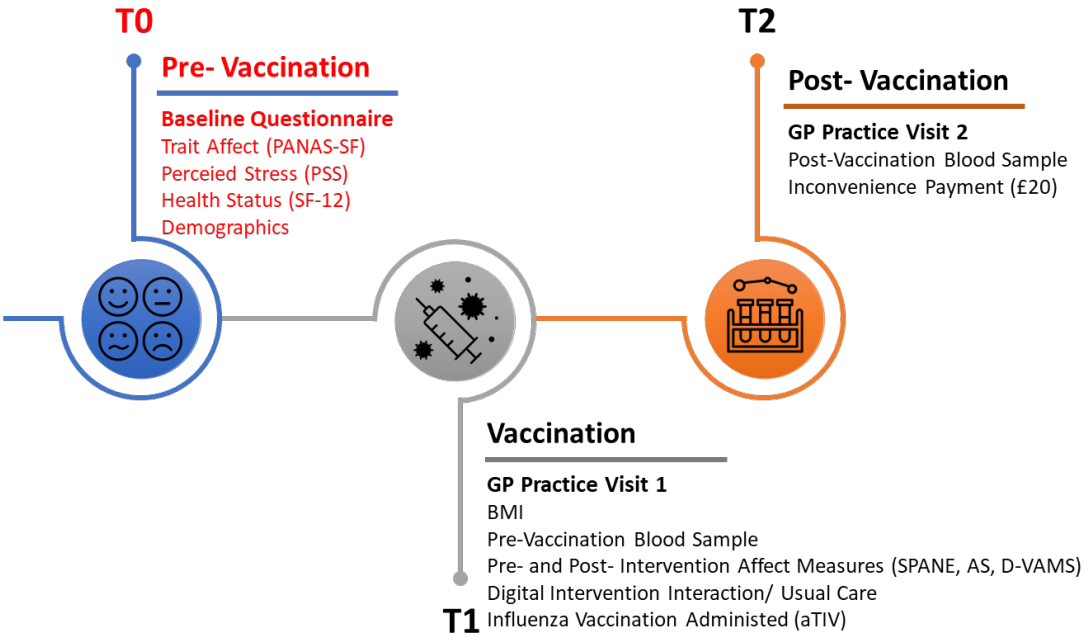



**Figure 5.2**: For-ME trial diagram highlighting pre-vaccination, baseline measures.

**Table 5.1:** For-ME participant demographic information collected at baseline.

| | **Whole Cohort** (*n*= 654) | **Usual Care** (*n*= 126) | **Standardised Intervention** (*n*= 264) | **Choice Intervention** (*n*= 264) |
|---|---|---|---|---|
| **Age (Years)** | | | | |
| Mean (SD) | 73.4 (5.10) | 74.0 (5.43) | 73.2 (5.11) | 73.3 (4.91) |
| Median [Min- Max] | 72.6 [65- 85] | 74.1 [65- 85] | 72.3 [65-85] | 72.5 [65-85] |
| **Gender** | | **Frequency (%)** | | |
| Male | 303 (46.3) | 59 (46.8) | 127 (48.1) | 117 (44.3) |
| Female | 351 (53.7) | 67 (53.2) | 137 (51.9) | 147 (55.7) |
| **Ethnicity** | | | | |
| White | 630 (96.3) | 121 (96.0) | 254 (96.2) | 255 (96.6) |
| Black | 2 (0.3) | 0 (0) | 2 (0.8) | 0 (0) |
| Asian | 3 (0.5) | 2 (1.6) | 1 (0.4) | 0 (0) |
| Mixed | 1 (0.2) | 0 (0) | 1 (0.4) | 0 (0) |
| Other | 1 (0.2) | 0 (0) | 1 (0.4) | 0 (0) |
| Missing | 17 (2.6) | 3 (2.4) | 5 (1.9) | 9 (3.4) |
| **Marital Status** | | | | |
| Married | 422 (64.5) | 79 (62.7) | 173 (65.5) | 170 (64.4) |
| Single, never married | 28 (4.3) | 9 (7.1) | 8 (3.0) | 11 (4.2) |
| Separated/ divorced | 74 (11.3) | 10 (7.9) | 35 (13.3) | 29 (11.0) |
| Widowed | 94 (14.4) | 19 (15.1) | 36 (13.6) | 39 (14.8) |
| Co-habiting | 21 (3.2) | 6 (4.8) | 8 (3.0) | 7 (2.7) |
| Missing | 15 (2.3) | 3 (2.4) | 4 (1.5) | 8 (3.0) |
| **Lives Independently** | | | | |
| No | 29 (4.4) | 6 (4.8) | 13 (4.9) | 10 (3.8) |
| Yes | 611 (93.4) | 117 (92.9) | 247 (93.6) | 247 (93.6) |
| Missing | 14 (2.1) | 3 (2.4) | 4 (1.5) | 7 (2.7) |
| **Highest Level of Education** | | | | |
| School | 313 (47.9) | 67 (53.2) | 119 (45.1) | 127 (48.1) |
| University (Undergraduate) | 69 (10.6) | 17 (13.5) | 27 (10.2) | 25 (9.5) |
| University (Postgraduate) | 115 (17.6) | 20 (15.9) | 45 (17.0) | 50 (18.9) |
| Other | 141 (21.6) | 19 (15.1) | 68 (25.8) | 54 (20.5) |
| Missing | 16 (2.4) | 3 (2.4) | 5 (1.9) | 8 (3.0) |
| **Current Smoker** | | | | |
| No | 608 (93.0) | 117 (92.9) | 251 (95.1) | 240 (90.9) |
| Yes | 32 (4.9) | 6 (4.8) | 9 (3.4) | 17 (6.4) |
| Missing | 14 (2.1) | 3 (2.4) | 4 (1.5) | 7 (2.7) |
| **BMI** | | | | |
| Mean (SD) | 27.7 (5.17) | 28.1 (5.76) | 27.2 (4.95) | 27.9 (5.08) |
| Median [Min- Max] | 27.0 [15.7- 51.8] | 27.3 [17.7- | 26.6 [15.7- 46.1] | 27.1 [17.5- |
| | 3 (0.5%) | 46.6] | 1 (0.4%) | 51.8] |
| Missing | | 1 (0.8%) | | 1 (0.4%) |

SD = Standard Deviation, BMI = Body Mass Index.

### *5.3.3 Baseline Health, Stress and Mood Characteristics*

*Table 5.2* presents an overview of participant mental health, physical health, perceived stress and trait mood characteristics measured at baseline. On average, the cohort demonstrated a low perceived stress score (mean = 12.5), whereby scores of 0-13 = low, 14-26 = moderate and 27-40 = high (Cohen et al., 1983). This is consistent with existing normative data presented for perceived stress of older adults (mean = 12.0) (Cohen & Williamson, 1988).

PANAS-SF scores range from 10-50, whereby this participant cohort had a mean trait positive affect score of 34.6 (SD= 7.7) and a mean trait negative affect score of 15.0 (SD= 5.9). The cohort PANAS-SF positive affect is slightly lower than normative data presented for this scale (mean = 38.4, SD = 4.9) and PANAS-SF negative affect is much lower (mean = 25.9, SD = 5.2) (Thompson, 2007). However, these normative data looked at young adult populations (<25 to >30 years), which could explain these observed differences. In contrast, another study conducted on an older adult population has indicated similar positive affect (mean= 33.5, SD= 8.6) and negative affect scores (mean= 15.2, SD= 6.7) to those observed in the For-ME trial (Ayling et al., 2019).

Participant health status defined by mean physical component summary (PCS) and mental component summary (MCS) scores were 45.7 and 53.3 respectively, which indicate poor physical health (defined by a score of 50 or less) and poor mental health (where a score of 42 or less may be indicative of 'clinical depression') (Ware et al., 2002). The PCS mean score is consistent with general population scores (mean = 45.3) (Herrman et al., 2002), as well as general population mean MCS scores (mean = 52.1) (A. W. Taylor et al., 2000).

**Table 5.2**: Participant baseline perceived stress, trait affect and health measures.

| | **Whole Cohort** | **Usual Care** | **Standardised Intervention** | **Choice Intervention** |
|---|---|---|---|---|
| | | **Mean (SD)** | | |
| **Perceived Stress (PSS)** | 12.5 (7.2) | 12.34 (7.0) | 12.6 (7.2) | 12.6 (7.3) |
| **PANAS-SF** | | | | |
| Trait Positive Affect | 34.6 (7.7) | 34.1 (7.9) | 34.8 (7.6) | 34.7 (7.7) |
| Trait Negative Affect | 15.0 (5.9) | 15.2 (5.7) | 15.0 (6.1) | 15.0 (5.8) |
| **Health Status** | | | | |
| Physical Health (PCS) | 45.7 (11.1) | 43.5 (11.1) | 47.0 (10.7) | 45.5 (11.4) |
| Mental Health (MCS) | 53.3 (9.2) | 53.0 (10.3) | 53.3 (8.8) | 53.4 (9.3) |

SD = Standard Deviation, PSS= Perceived Stress Score, PANAS-SF = Positive and Negative Affect Scale Short Form, PCS = Physical Component Summary, MSC = Mental Component Summary.

#### *5.3.3.1 Baseline Correlations*

*Table 5.3* presents the bivariate correlations between psychological factors and mental and physical health measured at baseline. PANAS-SF positive and negative affect scores showed a small but significant inverse correlation, which is in line with previous findings (Watson, Clark, & Tellegen, 1988). Perceived stress measures were strongly positively associated with PANAS-SF negative affect, strongly negatively associated with PANAS-SF positive affect and SF-12 mental health scores and showed a small but significant negative correlation with SF-12 physical health scores. PANAS-SF positive affect scores were positively associated with SF-12 mental and physical health scores. PANAS-SF negative scores were strongly positively associated with SF-12 mental health scores, and showed a small but significant negative correlation with SF-12 physical health scores. No significant associations were found between SF-12 physical and mental health scores. Significant associations observed between perceived stress, PANAS-SF positive and PANAS-SF negative are important to note to ensure later analyses take this into consideration.

**Table 5.3:** Correlations (Pearson's) between psychological factors and physical and mental health measured at baseline.

| | **1** | **2** | **3** | **4** | **5** |
|---|---|---|---|---|---|
| **(1) Perceived Stress** | - | | | | |
| **(2) PANAS-SF Positive** | **-0.478*** ** | - | | | |
| **(3) PANAS-SF Negative** | **0.745*** ** | **-0.352*** ** | - | | |
| **(4) Physical Health** | **-0.284*** ** | **0.301*** ** | **-0.170*** ** | - | |
| **(5) Mental Health** | **-0.708*** ** | **0.518*** ** | **0.718*** ** | 0.036 | - |

*$p$<.05, **$p$<.01, ***$p$<.001

## 5.4 Research Aims

### *5.4.1 Primary Aim: To assess whether a standardised and/or choice intervention could improve the positive mood of older adults, compared with usual care, and to identify which intervention type was superior.*

#### *5.4.1.1 Research question*

##### 5.4.1.1.1 Were there significant differences between the standardised, choice and usual care groups in terms of improving positive mood in older adults?

This section addresses the primary aim of the For-ME trial, which was to determine whether a standardised and/or choice intervention were capable of improving positive mood of older adults immediately prior to influenza vaccination compared with usual care, with the Affective Slider happiness scale as the primary outcome measure. This section also aimed to assess whether a standardised or choice intervention was superior at enhancing positive mood.

###### 5.4.1.1.1.1 Hypothesis

Based on previous research (Ayling et al., 2019), it was hypothesised that participants receiving the positive mood intervention(s) would show greater improvements in positive affect scores compared to usual care (primary outcome was the Affective Slider Happiness Subscale). Evidence has suggested that both autonomy and perceived control are linked to wellbeing and positive mood (Reis et al., 2000; Sheldon et al., 1996), as well as being an important component in behaviour change (Terry & O'Leary, 1995). Given that choice may increase both perceived control and autonomy, it is possible that giving individuals an element of choice could lead to changes in both behaviour and mood. It was therefore hypothesised that the choice intervention would be superior to the standardised intervention in terms of predicting higher post-intervention positive state affect scores. Analyses were also carried out to assess whether any changes in mood from pre- to post-intervention were statistically significant.

###### 5.4.1.1.1.2 Analyses

*Figure 5.3* indicates (in red) when pre- and post-intervention state affect measures were obtained as part of the For-ME trial. *Table 5.4* presents an overview of participant pre- and post-intervention mood characteristics. Whole cohort and trial arm averages were similar with state affect scores for all affect measures increasing from pre- to post-intervention. Whole cohort Affective Slider scores of happiness and alertness were high (happiness pre- mean = 78.6, happiness post- mean = 84.4, alertness pre- mean = 82.9, alertness post- mean

= 86.6), where scores can range from 0-100. Pre- (mean = 22.3) and post- (mean = 24.0) intervention SPANE positive affect scores were moderate and pre- (mean = 7.6) and post- (mean = 7.0) intervention SPANE negative affect scores were very low for the whole cohort, where 6-30 is the scoring range for these scales. Similar to the Affective Slider, DVAMS measures happiness and alertness on a scale of 0-100. DVAMS scores were high pre- (happiness mean = 77.9, alertness mean = 77.2) and post- (happiness mean = 84.0, and alertness mean = 82.0) intervention for the whole cohort.

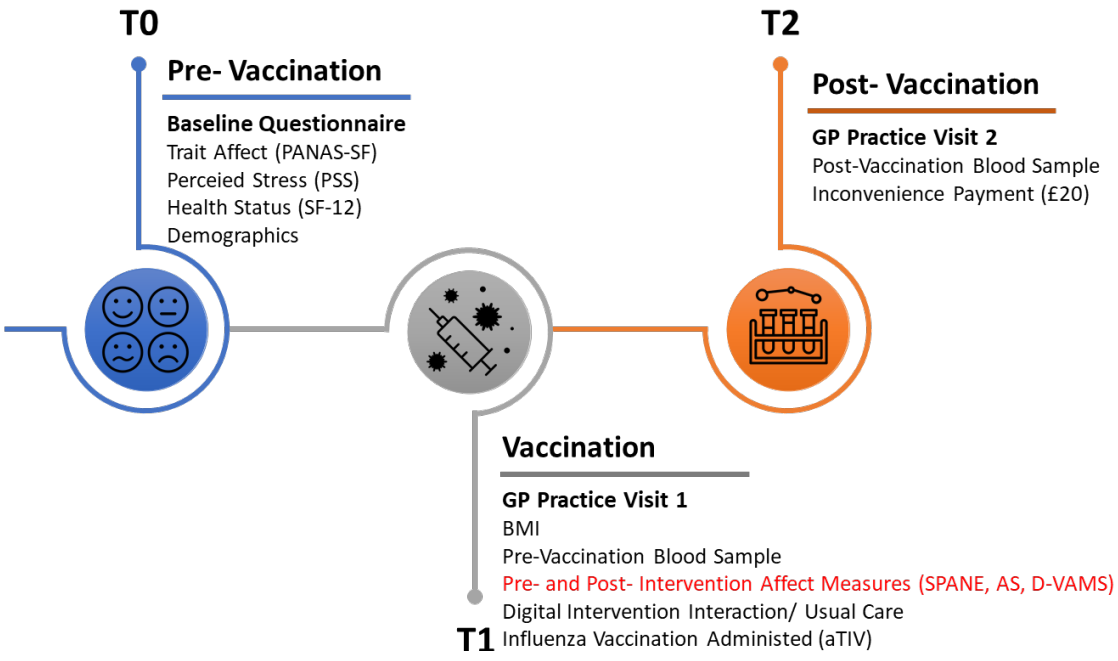



**Figure 5.3**: For-ME trial diagram highlighting pre- and post-intervention affect measures.

**Table 5.4**: Participant pre- and post-intervention affect measures.

| | Whole Cohort | Usual Care | Standardised Intervention | Choice Intervention |
|---|---|---|---|---|
| | | Mean (SD) | | |
| **Affective Slider** | | | | |
| Happiness (Pre) | 78.6 (20.9) | 76.8 (23.4) | 78.2 (21.4) | 79.9 (19.0) |
| Happiness (Post) | 84.4 (18.8) | 79.1 (22.4) | 84.8 (18.7) | 86.6 (16.5) |
| Alertness (Pre) | 82.9 (18.3) | 81.8 (21.0) | 83.4 (16.2) | 82.9 (18.9) |
| Alertness (Post) | 86.6 (16.4) | 83.4 (20.3) | 86.9 (15.4) | 88.0 (15.2) |
| **SPANE** | | | | |
| Positive Affect (Pre) | 22.3 (4.9) | 22.0 (5.2) | 22.1 (5.0) | 22.5 (4.6) |
| Positive Affect (Post) | 24.0 (4.9) | 22.7 (5.1) | 23.9 (5.0) | 24.7 (4.5) |
| Negative Affect (Pre) | 7.6 (2.9) | 8.1 (3.3) | 7.5 (2.6) | 7.5 (2.8) |
| Negative Affect (Post) | 7.0 (2.4) | 7.8 (3.0) | 6.8 (2.0) | 6.8 (2.3) |
| **DVAMS** | | | | |
| Happiness (Pre) | 77.9 (17.3) | 76.8 (19.2) | 77.2 (17.4) | 79.2 (16.2) |
| Happiness (Post) | 84.0 (16.5) | 78.3 (19.4) | 84.4 (16.8) | 86.3 (14.0) |
| Alertness (Pre) | 77.2 (16.9) | 76.2 (19.8) | 76.9 (16.1) | 78.1 (16.2) |
| Alertness (Post) | 82.0 (16.0) | 78.4 (18.3) | 82.0 (15.7) | 83.9 (14.6) |

SD = Standard Deviation, Pre = pre-intervention, Post = post-intervention, SPANE = Scale of Positive and Negative Experience, DVAMS = Dynamic Visual Analogue Mood Scales.

*Figure 5.4* shows the change in pre- to post-intervention mood mean scores measured using the happiness Affective Slider (primary outcome measure), alertness Affective Slider, happiness DVAMS, alertness DVAMS, SPANE positive and SPANE negative for each trial arm of the population. These bar graphs illustrated a greater increase in mean scores for standardised and choice intervention groups compared with usual care groups for both Affective Slider scales (happiness subscale being the primary outcome measure). Additionally, a greater increase in both DVAMS and positive SPANE, and a greater decrease in negative SPANE from pre- to post-intervention was observed for both intervention groups compared with usual care. Paired t test analyses indicated that these changes from pre- to post-intervention for both intervention groups and the usual care group were statistically significant (except of the DVAMS happiness subscale).

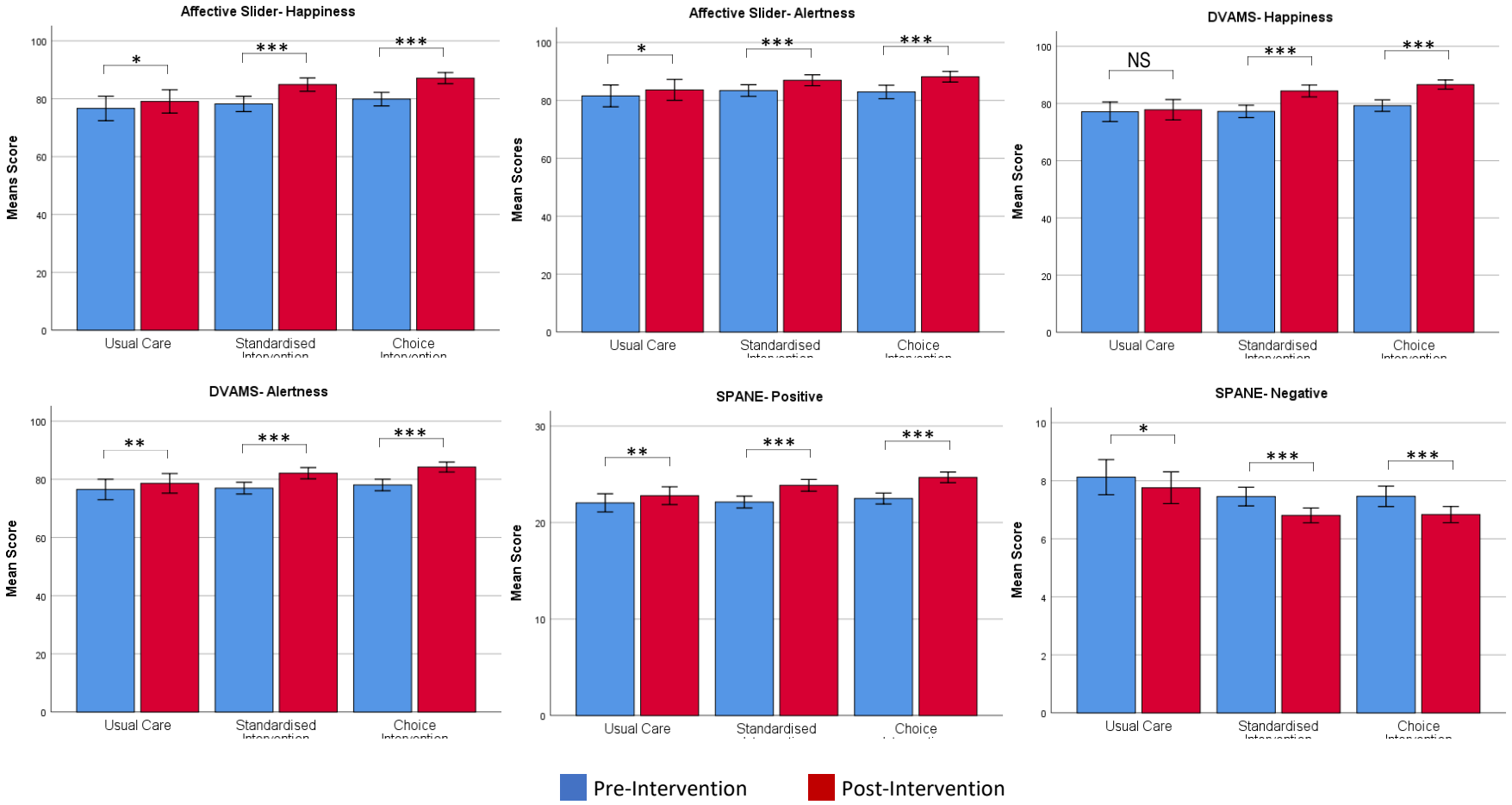



**Figure 5.4**: Mood measures (Affective Slider happiness and alertness, DVAMS happiness and alertness, SPANE positive and SPANE negative) for all trial arms, highlighting the mean score change from pre- to post-intervention. Error bars indicating 95% confidence interval. *$p$<.05, **$p$<.01, ***$p$<.001, NS= $p \geq 0.05$.

To assess if there were statistically significant differences in post- intervention scores between groups, a series of one-way ANCOVA's were performed with pre-intervention scores (for each measure) included as a covariate. These demonstrated that there was a significant effect of group allocation on mood scores for all of the six measured outcomes (see *Table 5.5*. Affective Slider happiness $F$ (2,631) = 9.532, $p$<0.001, Affective Slider alertness $F$ (2,631) = 5.462, $p$=0.004, DVAMS happiness $F$ (2,619) = 20.964, $p$<0.001, DVAMS alertness $F$ (2,619) = 8.689, $p$<0.001, SPANE positive $F$ (2,629) = 11.016, $p$<0.001, SPANE negative $F$ (2,627) = 5.386, $p$=0.005). Effect sizes (partial Eta squared) were small to medium for these comparisons.

**Table 5.5**: One-way ANCOVA comparisons of mood outcomes for usual care, standardised intervention and choice intervention groups.

| | Degrees of Freedom | F value | P value | Partial Eta Squared |
|---|---|---|---|---|
| **Affective Slider Happiness** | 2 | 9.532 | **<0.001*** ** | 0.029 |
| **Affective Slider Alertness** | 2 | 5.462 | **0.004** ** | 0.017 |
| **DVAMS Happiness** | 2 | 20.964 | **<0.001*** ** | 0.063 |
| **DVAMS Alertness** | 2 | 8.689 | **<0.001*** ** | 0.027 |
| **SPANE Positive** | 2 | 11.016 | **<0.001*** ** | 0.034 |
| **SPANE Negative** | 2 | 5.386 | **0.005** ** | 0.017 |

*$p$<.05, **$p$<.01, ***$p$<.001

Further analyses were conducted to assess pairwise comparisons and investigate differences between each of the three allocation groups to determine whether one of the intervention types was superior (see *Table 5.6*). There were significant differences between the standardised intervention and usual care groups for all state affect outcome measures with the exception of the alertness Affective Slider ($p$= 0.064). There were also significant differences between the choice intervention and usual care groups for all state affect outcome measures. However, there were no statistically significant differences between the standardised and choice groups. This indicated that while both intervention types were capable of significantly increasing positive affect and significantly decreasing negative affect compared with usual care, neither intervention type was significantly superior.

**Table 5.6:** Estimated marginal means and pairwise comparisons of post-intervention mood outcomes for each trial arm (standardised intervention, choice intervention and usual care).

| | Group Allocation | EM Means (SE) | 95% CI (lower-upper) | Pairwise Comparison Groups | Mean Difference (SE) | P value |
|---|---|---|---|---|---|---|
| **Affective Slider Happiness** | Usual Care | 80.28 (1.16) | 78.0-82.5 | **UC v ST** | 4.84 (1.40) | **0.001*** ** |
| | Standardised | 85.12 (0.80) | 83.6-86.7 | **UC v CH** | 6.04 (1.40) | **<.001*** ** |
| | Choice | 86.32 (0.80) | 84.8-87.9 | **ST v CH** | 1.20 (1.13) | 0.287 |
| **Affective Slider Alertness** | Usual Care | 84.54 (0.91) | 82.8-86.3 | **UC v ST** | 2.04 (1.10) | 0.064 |
| | Standardised | 86.58 (0.63) | 87.8-89.4 | **UC v CH** | 3.59 (1.10) | **0.001*** ** |
| | Choice | 88.14 (0.62) | 86.9-89.4 | **ST v CH** | 1.55 (0.88) | 0.079 |
| **DVAMS Happiness** | Usual Care | 78.46 (0.97) | 76.6-80.4 | **UC v ST** | 6.49 (1.17) | **<.001*** ** |
| | Standardised | 84.95 (0.66) | 83.6-86.4 | **UC v CH** | 7.32 (1.17) | **<.001*** ** |
| | Choice | 85.78 (0.66) | 84.5-87.1 | **ST v CH** | 0.83 (0.94) | 0.377 |
| **DVAMS Alertness** | Usual Care | 79.20 (0.89) | 77.4-80.9 | **UC v ST** | 3.15 (1.08) | **0.003** ** |
| | Standardised | 82.35 (0.61) | 81.2-83.5 | **UC v CH** | 4.48 (1.08) | **<.001*** ** |
| | Choice | 83.68 (0.61) | 82.5-84.9 | **ST v CH** | 1.33 (0.86) | 0.122 |
| **SPANE Positive** | Usual Care | 22.95 (0.27) | 22.4-23.5 | **UC v ST** | 1.01 (0.33) | **0.002** ** |
| | Standardised | 23.96 (0.19) | 23.6-24.3 | **UC v CH** | 1.56 (0.33) | **<.001*** ** |
| | Choice | 24.51 (0.19) | 24.1-24.9 | **ST v CH** | 0.55 (0.27) | **0.040*** |
| **SPANE Negative** | Usual Care | 7.44 (0.15) | 7.2-7.7 | **UC v ST** | 0.56 (0.18) | **0.002** ** |
| | Standardised | 6.89 (0.10) | 6.7-7.1 | **UC v CH** | 0.54 (0.18) | **0.003** ** |
| | Choice | 6.91 (0.10) | 6.7-7.1 | **ST v CH** | 0.02 (0.15) | 0.875 |

SE = Standard Error, EM = Estimated Marginal, CI = Confidence Interval, UC = Usual Care, ST = Standardised, CH = Choice. *$p$<.05, **$p$<.01, ***$p$<.001

#### 5.4.1.1.1.3 Summary of Results

Change in state affect, measured by Affective Slider scales, DVAMS and SPANE, from pre- to post-intervention were found to be statistically significant for both intervention groups, for all outcome measures ($p$<0.001). This indicated that both interventions successfully enhanced the positive mood, and decreased the negative mood, of older adults immediately prior to influenza vaccination. Some statistically significant changes were also observed in the usual care arm for both Affective Slider scales, both SPANE scales and the alertness DVAMS scale ($p$<.05 and $p$<.01), except for the DVAMS happiness subscale. However, ANCOVA analyses supported the hypothesis that both interventions would increase positive mood more than usual care. The analyses presented in this section indicated that group allocation to an intervention group, standardised or choice, was a significant predictor of post- intervention state affect compared with usual care. However, in contrast to the outlined hypothesis, the choice intervention was not shown to be significantly superior at predicting post- intervention state positive affect scores compared with the no choice, standardised (fixed-content) intervention.

### *5.4.2 Secondary Aim: To assess whether there were differences in 4-weeks post-vaccination responses to influenza vaccination between the standardised, choice and usual care groups.*

#### *5.4.2.1 Research Question*

##### 5.4.2.1.1 Were there significant differences between the standardised, choice and usual care groups in terms of 4-weeks post-vaccination antibody response to each influenza vaccine strain?

This section addresses the secondary aim of the For-ME trial to investigate whether trial arm allocation predicted significant differences between antibody responses to each influenza vaccination strain.

##### 5.4.2.1.1.1 Hypothesis

Previous research (discussed in *Chapter 2*) has outlined that negative and positive emotional experiences are capable of regulating immunity, whereby positive mood has been identified as having immune enhancing effects. For this reason, it was hypothesised that participants randomised to an intervention arm, a positive mood enhancing intervention, were more likely to present greater post-vaccination antibody levels, in terms of ELISA OD levels and in terms of the proportion of participants classified as seroprotected, compared with participants randomised to usual care. It was also hypothesised that statistically significant changes in antibody levels and seroprotection would be observed from pre- to 4-weeks post-vaccination, which would highlight the efficacy of the administered vaccine.

##### 5.4.2.1.1.2 Analyses

*Figure 5.5* outlines (in red) participant pre- and 4-weeks post-vaccination blood sample collection as part of the For-ME trial to measure antibody responses to vaccination. Two different laboratory techniques were utilised to measure antibody responses to vaccination: Enzyme-linked Immunosorbent Assay (ELISA) and Hemagglutination Inhibition (HAI) Assay. *Table 5.7* presents an overview of the pre- and 4-weeks post-vaccination antibody levels, assessed via ELISA and HAI. This table highlights both the geometric mean (GeoM) titres measured by HAI and the proportion of participants classified as seroprotection (defined by meeting a GeoM threshold of ≥40 and ≥160).

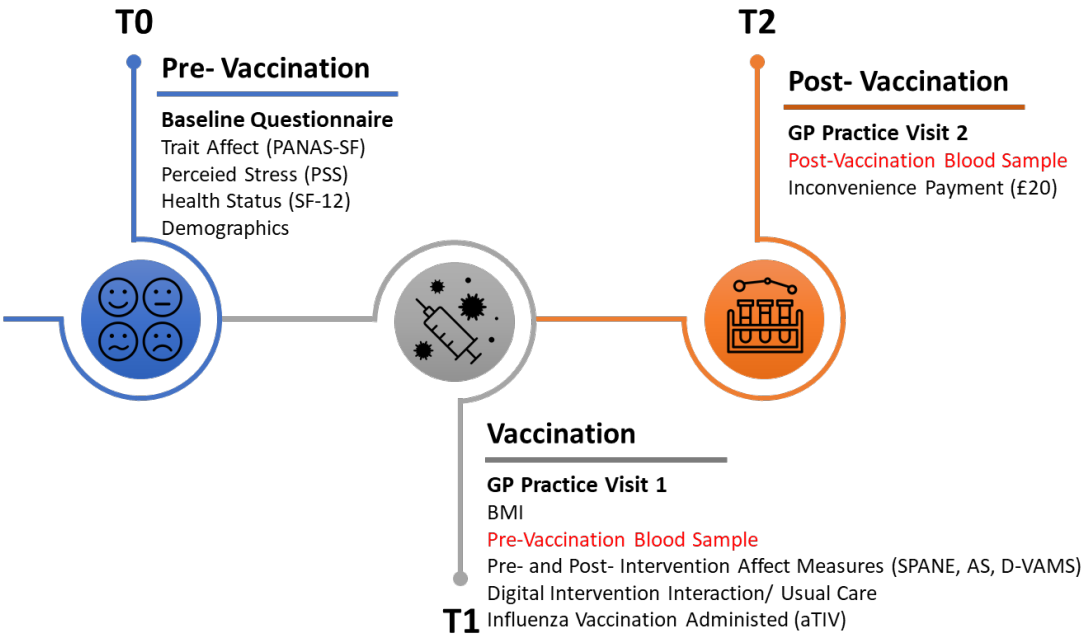



**Figure 5.5**: For-ME trial design highlighting pre- and 4-weeks post-vaccination blood sample collection to measure influenza vaccination responses.

**Table 5.7:** Participant antibody levels pre- and post-vaccination measured by ELISA and HAI.

| | Whole Cohort | Usual Care | Standardised Intervention | Choice Intervention |
|---|---|---|---|---|
| | | **Mean (SD)** | | |
| **ELISA IgG (OD)** | | | | |
| A/Brisbane/02/2018 (H1N1) (Pre) | 0.91 (0.34) | 0.94 (0.37) | 0.90 (0.29) | 0.74 (0.34) |
| A/Brisbane/02/2018 (H1N1) (Post) | 1.17 (0.41) | 1.19 (0.42) | 1.19 (0.42) | 1.16 (0.47) |
| A/Kansas/14/2017 (H3N2) (Pre) | 0.76 (0.34) | 0.81 (0.38) | 0.76 (0.31) | 0.75 (0.44) |
| A/Kansas/14/2017 (H3N2) (Post) | 1.20 (0.48) | 1.20 (0.51) | 1.23 (0.47) | 1.16 (0.47) |
| B/Colorado/06/2017-like (B) (Pre) | 1.30 (0.52) | 1.38 (0.53) | 1.28 (0.50) | 1.26 (0.53) |
| B/Colorado/06/2017-like (B) (Post) | 1.62 (0.53) | 1.66 (0.56) | 1.63 (0.55) | 1.58 (0.49) |
| **HAI Titres (GeoM)** | | | | |
| A/Brisbane/02/2018 (H1N1) (Pre) | 126.5 (220.7) | 130.6 (226.7) | 114.0 (200.0) | 137.0 (237.3) |
| A/Brisbane/02/2018 (H1N1) (Post) | 305.9 (363.1) | 319.0 (371.5) | 295.1 (354.5) | 310.3 (368.5) |
| A/Kansas/14/2017 (H3N2) (Pre) | 49.7 (112.8) | 54.2 (129.9) | 61.6 (142.6) | 35.7 (54.7) |
| A/Kansas/14/2017 (H3N2) (Post) | 429.9 (449.4) | 442.7 (460.6) | 444.5 (462.2) | 409.0 (431.5) |
| B/Colorado/06/2017 (B) (Pre) | 220.2 (314.3) | 268.4 (379.2) | 209.9 (301.2) | 207.9 (292.0) |
| B/Colorado/06/2017 (B) (Post) | 345.1 (368.0) | 389.7 (416.5) | 332.1 (352.0) | 335.9 (357.6) |
| | | | **Frequency (%)** | |
| **Seroprotection (titre ≥40)** | | | | |
| A/Brisbane/02/2018 (H1N1) (Pre) | 406 (63.7%) | 75 (62.0%) | 169 (65.8%) | 162 (62.5%) |
| A/Brisbane/02/2018 (H1N1) (Post) | 541 (89.7%) | 109 (90.8%) | 218 (90.5%) | 214 (88.4%) |
| A/Kansas/14/2017 (H3N2) (Pre) | 188 (29.5%) | 30 (24.8%) | 86 (33.5%) | 72 (27.8%) |
| A/Kansas/14/2017 (H3N2) (Post) | 533 (88.4%) | 106 (88.3%) | 213 (88.4%) | 214 (88.4%) |
| B/Colorado/06/2017 (B) (Pre) | 509 (79.9%) | 94 (77.7%) | 211 (82.1%) | 204 (78.8%) |
| B/Colorado/06/2017 (B) (Post) | 577 (95.7%) | 116 (96.7%) | 229 (95.0%) | 232 (95.9%) |
| **Seroprotection (titre ≥160)** | | | | |
| A/Brisbane/02/2018 (H1N1) (Pre) | 190 (29.1%) | 37 (29.4%) | 73 (27.7%) | 80 (30.3%) |
| A/Brisbane/02/2018 (H1N1) (Post) | 416 (63.6%) | 79 (62.7%) | 167 (63.3%) | 170 (64.4%) |
| A/Kansas/14/2017 (H3N2) (Pre) | 76 (11.6%) | 17 (13.5%) | 35 (13.3%) | 24 (9.1%) |
| A/Kansas/14/2017 (H3N2) (Post) | 436 (66.7%) | 82 (65.1%) | 180 (68.2%) | 174 (65.9%) |
| B/Colorado/06/2017 (B) (Pre) | 286 (43.7%) | 60 (47.6%) | 109 (41.3%) | 117 (44.3%) |
| B/Colorado/06/2017 (B) (Post) | 439 (67.1%) | 82 (65.1%) | 178 (67.2%) | 179 (67.8%) |

SD = Standard Deviation, ELISA = Enzyme Linked Immunosorbent Assay, OD = Optical Density, HAI = Hemagglutinin Inhibition, GeoM = Geometric Mean, Pre = Pre-vaccination, Post = post-vaccination.

#### *5.4.2.1.1.2.1 Enzyme- Linked Immunosorbent Assay*

Pre-vaccination, mean ELISA OD values were 0.91, 0.76 and 1.30 and post-vaccination were 1.17, 1.20 and 1.62 for H1N1, H3N2 and B strains, respectively. Paired t-test analyses indicated that these changes in antibody levels from pre- to post-vaccination were statistically significant (see *Figure 5.6*).

One-way ANCOVAs were conducted, with pre-vaccination values selected as the covariate and post-vaccination values as the dependent variable, to determine whether intervention group allocation influenced antibody responses to vaccination. These analyses indicated that group allocation did not have a statistically significant influence on post-vaccination IgG influenza-specific antibody levels as measured by ELISA (see *Table 5.8*, A/Brisbane/02/2018 (H1N1) $F$ (2,584) = 0.706, $p$=0.494, A/Kansas/14/2017 (H3N2) $F$ (2,584) = 0.901, $p$= 0.407, B/Colorado/06/2017 (B) $F$ (2,584) = 0.206, $p$= 0.814). *Figure 5.7* illustrates the estimated marginal means for each influenza vaccine strain, highlighting the similar post-vaccination responses between the standardised, choice and usual care groups.

Whilst there were no observed differences of group allocation on IgG antibody responses to influenza vaccination measured by ELISA, pre-planned analyses were conducted to take a closer look at pairwise comparisons of the trial groups for each vaccine component (see *Table 5.9*). Consistent with the above, pairwise comparisons of estimated marginal means indicated no significant differences between standardised and usual care groups (A/Brisbane/02/2018 (H1N1) $p$= 0.988, A/Kansas/14/2017 (H3N2) $p$= 0.293, B/Colorado/06/2017-like (B) $p$= 0.529), choice and usual care groups (A/Brisbane/02/2018 (H1N1) $p$= 0.377, A/Kansas/14/2017 (H3N2) $p$= 0.921, B/Colorado/06/2017-like (B) $p$= 0.734) or standardised and choice groups (A/Brisbane/02/2018 (H1N1) $p$= 0.281, A/Kansas/14/2017 (H3N2) $p$= 0.235, B/Colorado/06/2017-like (B) $p$= 0.717) in terms of ELISA OD levels.

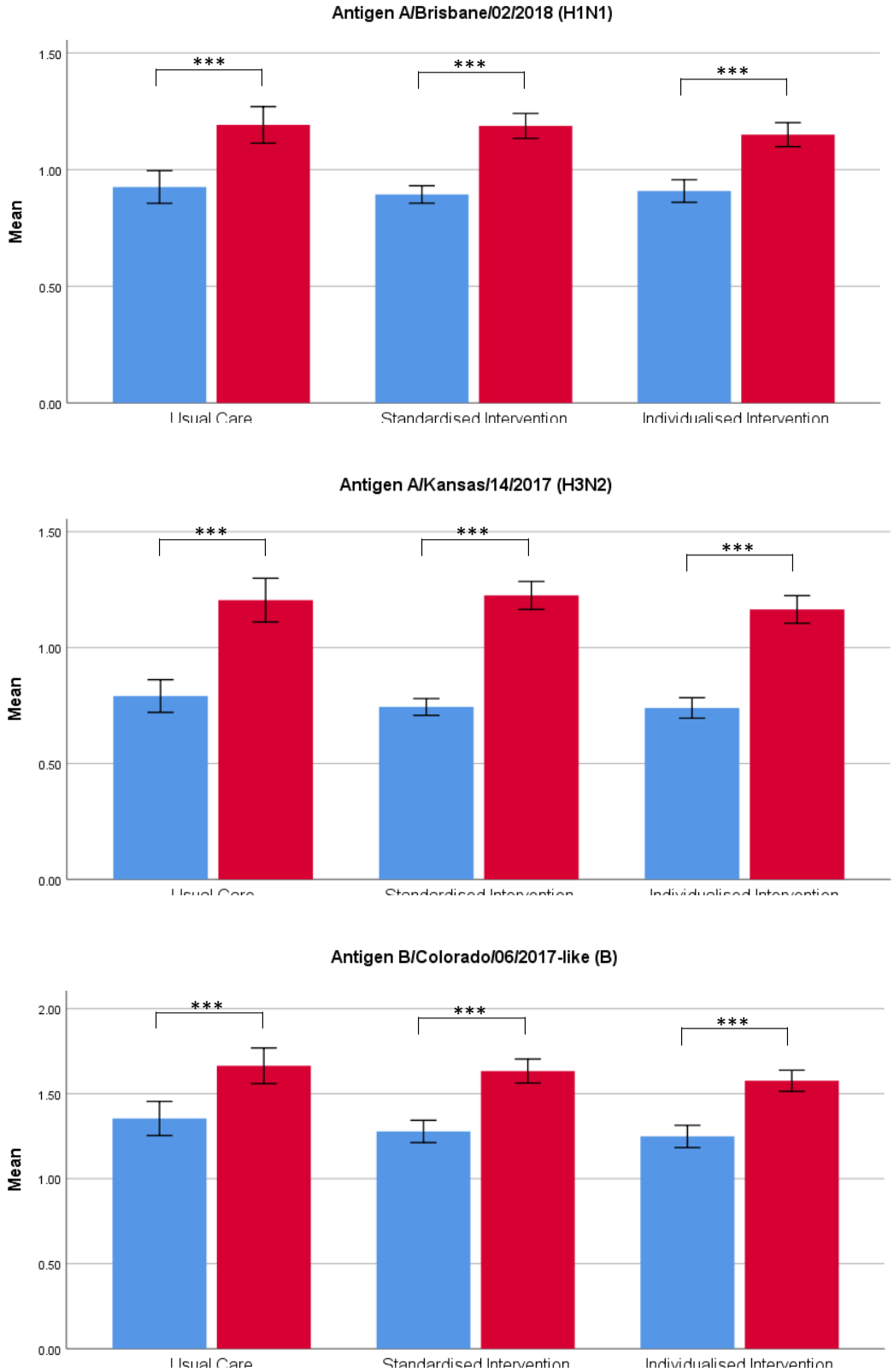



**Figure 5.6**: Pre- and post-vaccination antibody responses to influenza vaccination measured by ELISA for each trial arm (standardised intervention, choice intervention and usual care). Error bars indicate 95% confidence interval. **p*<.05, ***p*<.01, ****p*<.001.

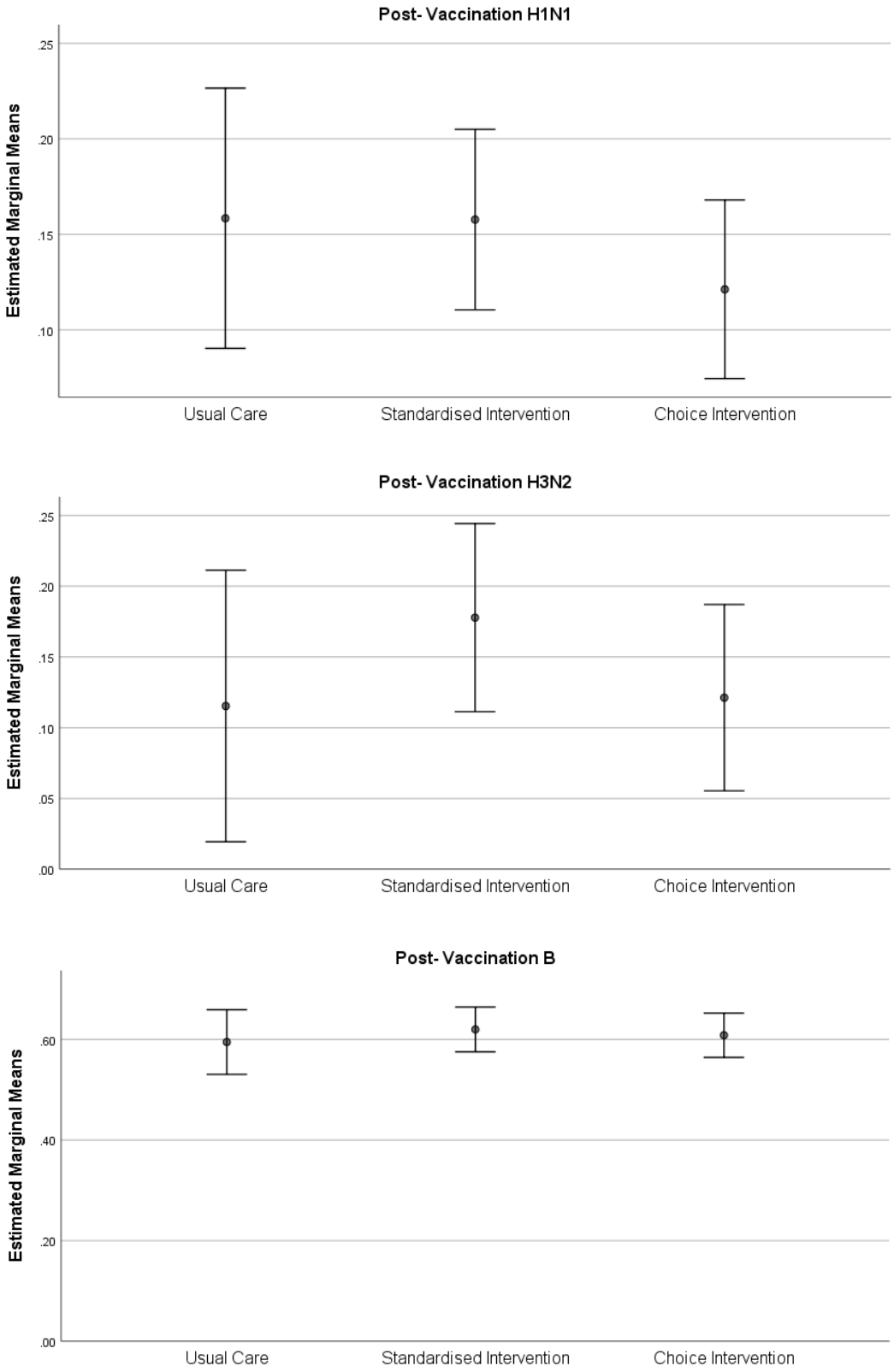



**Figure 5.7**: Estimated marginal means of post-vaccination influenza strains (H1N1, H3N2 and B) for usual care, standardised intervention and choice intervention groups. Error bars indicating 95% confidence intervals.

**Table 5.8**: One-way ANCOVA comparison of ELISA IgG levels between trial groups (standardised intervention, choice intervention and usual care).

| | Degrees of Freedom | F value | p value | Partial Eta Squared |
|---|---|---|---|---|
| **A/Brisbane/02/2018 (H1N1)** | 2 | 0.706 | 0.494 | 0.002 |
| **A/Kansas/14/2017 (H3N2)** | 2 | 0.901 | 0.407 | 0.003 |
| **B/Colorado/06/2017-like (B)** | 2 | 0.206 | 0.814 | 0.001 |

*$p$<.05, **$p$<.01, ***$p$<.001 (no statistical significance identified in this table).

**Table 5.9**: Estimated marginal means and pairwise comparisons of post-vaccination IgG levels for each trial arm (standardised intervention, choice intervention and usual care).

| | Group Allocation | EM Means (SE) | 95% CI (lower-upper) | Pairwise Comparison Groups | Mean Difference (SE) | *p* value |
|---|---|---|---|---|---|---|
| **A/Brisbane/02/2018 (H1N1)** | Usual Care | 0.16 (0.04) | 0.09-0.23 | **UC v ST** | 0.001 (0.04) | 0.988 |
| | Standardised | 0.16 (0.02) | 0.11-0.21 | **UC v CH** | 0.037 (0.04) | 0.377 |
| | Choice | 0.12 (0.02) | 0.07-0.17 | **ST v CH** | 0.037 (0.04) | 0.281 |
| **A/Kansas/14/2017 (H3N2)** | Usual Care | 0.12 (0.05) | 0.02-0.21 | **UC v ST** | 0.062 (0.06) | 0.293 |
| | Standardised | 0.18 (0.03) | 0.11-0.24 | **UC v CH** | 0.006 (0.06) | 0.921 |
| | Choice | 0.12 (0.03) | 0.06-0.19 | **ST v CH** | 0.06 (0.05) | 0.235 |
| **B/Colorado/06/2017-like (B)** | Usual Care | 0.60 (0.03) | 0.53-0.66 | **UC v ST** | 0.025 (0.04) | 0.529 |
| | Standardised | 0.62 (0.02) | 0.58-0.66 | **UC v CH** | 0.014 (0.04) | 0.734 |
| | Choice | 0.61 (0.02) | 0.56-0.65 | **ST v CH** | 0.012 (0.03) | 0.717 |

SE = Standard Error, EM = Estimated Marginal, CI = Confidence Interval, UC = Usual Care, ST = Standardised, CH = Choice. *$p$<.05, **$p$<.01, ***$p$<.001 (no statistical significance identified in this table).

#### *5.4.2.1.1.2.2 Hemagglutinin Inhibition Assay*

The proportion of participants classified as seroprotected (≥40) pre-vaccination were 64%, 30% and 80% for strains H1N1, H3N2 and B, respectively. Post-vaccination, these proportions increased to 90%, 88% and 96%. Considering the higher seroprotection threshold of ≥160, the proportion of participants meeting this criteria pre-vaccination was much lower with proportions of 30%, 12% and 44% for H1N1, H3N2 and B, respectively. Post- vaccination, these proportions increased to 64%, 67% and 67% for these vaccine components. McNamar analyses were conducted to assess pre- and post-vaccination seroprotection changes and were found to be statistically significant for all influenza strains for HAI thresholds ≥40 and ≥160 (see *Figure 5.8*).

*Figure 5.8* illustrates the proportion of participants who were classified as seroprotected (thresholds ≥40 and ≥160) post-vaccination compared with those who were not for each trial group. Chi-squared tests were conducted to examine whether there were significant differences in post-vaccination seroprotection rates (at thresholds ≥40 and ≥160) between study arms (see *Table 5.10*). These analyses indicated there is no significant differences in rates of seroprotection between arms for either threshold (A/Brisbane/02/2018 (H1N1) ≥40 $X^2$ (2, $N$ = 654) = 1.78, $p$ = 0.411, A/Kansas/14/2017 (H3N2) ≥40 $X^2$ (2, $N$ = 654) = 0.73, $p$ = 0.695, B/Colorado/06/2017 (B) ≥40 $X^2$ (2, $N$ = 654) = 2.38, $p$ = 0.305, A/Brisbane/02/2018 (H1N1) ≥160 $X^2$ (2, $N$ = 654) = 0.13, $p$ = 0.937, A/Kansas/14/2017 (H3N2) ≥160 $X^2$ (2, $N$ = 654) = 0.48, $p$ = 0.785, B/Colorado/06/2017 (B) ≥160 $X^2$ (2, $N$ = 654) = 0.31, $p$ = 0.859.

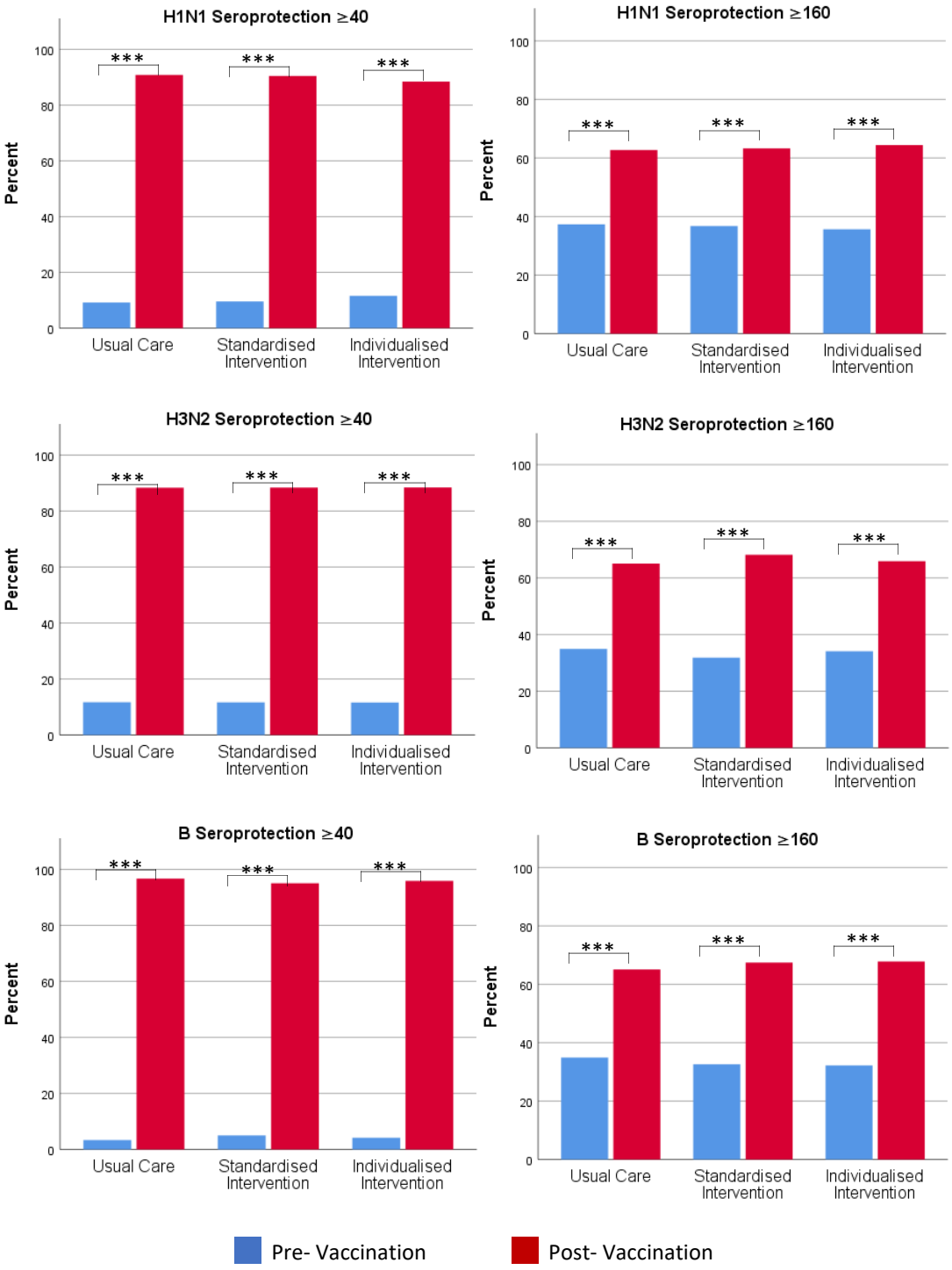



**Figure 5.8:** Proportion of participants classified as seroprotected (thresholds ≥40 and ≥160) and not seroprotected for each influenza vaccine component (H1N2, H3N2 and B), for each trial group (standardised intervention, choice intervention and usual care) post-vaccination.

**Table 5.10**: Chi-squared investigation of relationship between trial group allocation (standardised intervention, choice intervention and usual care) and post-vaccination HAI seroprotection threholds.

| | Degrees of Freedom | $X^2$ | *p* value | Phi and Cramer's V |
|---|---|---|---|---|
| **A/Brisbane/02/2018 (H1N1) ≥40 threshold** | 2 | 1.777 | 0.411 | 0.052 |
| **A/Kansas/14/2017 (H3N2) ≥40 threshold** | 2 | 0.728 | 0.695 | 0.033 |
| **B/Colorado/06/2017 (B) ≥40 threshold** | 2 | 2.376 | 0.305 | 0.060 |
| **A/Brisbane/02/2018 (H1N1) ≥160 threshold** | 2 | 0.129 | 0.937 | 0.014 |
| **A/Kansas/14/2017 (H3N2) ≥160 threshold** | 2 | 0.484 | 0.785 | 0.027 |
| **B/Colorado/06/2017 (B) ≥160 threshold** | 2 | 0.305 | 0.859 | 0.022 |

$X^2$ = Chi-squared statistic value. **p*<.05, ***p*<.01, ****p*<.001 (no statistical significance identified in this table).

#### 5.4.2.1.1.3 Summary of Results

The above findings indicate that across all groups, for all strains, there were statistically significant changes in antibody levels, measured by ELISA and HAI, following vaccination (all *p*<.001). In contrast to the outlined hypothesis, these results have indicated that group allocation did not significantly predict 4-week post-vaccination antibody responses to influenza vaccination in terms of ELISA OD levels or in terms of the proportion of participants classified as seroprotected for either threshold.

## 5.5 Exploratory Analyses

### *5.5.1 Exploratory Question 1: To assess whether perceived stress and trait mood were associated with positive mood on the day of vaccination.*

#### *5.5.1.1 To what extent did psychological factors at baseline influence pre-intervention positive mood on the day of vaccination?*

##### 5.5.1.1.1 Hypotheses

It was hypothesised that participants with high levels of perceived stress and negative trait affect measured at baseline would present lower pre-intervention positive state affect scores. It was also hypothesised that higher levels of positive trait affect would be associated with higher levels of pre-intervention positive state affect.

##### 5.5.1.1.2 Analyses

Multiple regression analyses were carried out to investigate whether perceived stress (PSS scale) and trait mood (PANAS-SF positive and PANAS-SF negative) measured at baseline were significant independent predictors of pre-intervention state mood (SPANE positive, SPANE negative, Affective Slider happiness, Affective Slider alertness, DVAMS happiness and DVAMS alertness) (see *Table 5.11- 5.16*). These analyses did not look at trial group allocation but instead investigated the whole cohort, which meant it was important to control for confounding factors. For each pre-intervention state affect measure, age and sex regression models were run to determine if they were significant predictive factors. Age and sex were controlled for, prior to the addition of other variables to the regression models (*Regression 1* highlighted in the tables) and were not found to be predictors of pre-intervention mood scores.

Due to the significant associations between the baseline measures (perceived stress and PANAS-SF positive and negative), outlined in *Section 5.3.3.1*, separate two-step regression models were run for each baseline variable (i.e., Step 1: age and sex, Step 2: perceived stress/ PANAS-SF positive/ PANAS-SF negative). This means a 4-step regression analyses was run for the following: *Regression 1*= age and sex, *Regression 2*= perceived stress, *Regression 3*= positive trait affect, *Regression 4*= negative trait affect.

For all pre-intervention mood measures, perceived stress and trait positive and negative affect (PANAS-SF positive and negative) were indicated as significant independent predictors. Greater perceived stress scores were associated with lower pre-intervention positive mood scores for the majority of measures (SPANE positive B= -0.330 (-0.379- -0.282), *β*= -0.487, $p<0.001$, Affective Slider happiness B= -1.359 (-1.564- -1.154), *β*= -0.476, $p<0.001$,

Affective Slider alertness B= -0.984 (-1.172- -0.796) *β*= -0.391, *p*<0.001, DVAMS happiness B= -1.146 (-1.318- -0.973), *β*= -0.479, *p*<0.001, DVAMS alertness (B= -0.891 (-1.067- -0.716) *β*= -0.383, *p*<0.001). In contrast, perceived stress was shown to be associated with elevated pre-intervention SPANE negative mood scores (B= 0.218 (0.190-0.246) *β*= 0.539, *p*<0.001).

Greater trait positive affect (PANAS-SF positive) scores were associated with higher pre-intervention mood score for the majority of measures (SPANE positive B= 0.323 (0.278-0.367), *β*= 0.512, *p*<0.001, Affective Slider happiness B= 1.094 (0.898-1.291), *β*= 0.414, *p*<0.001, Affective Slider alertness B= 1.022 (0.850-1.193), *β*= 0.437, *p*<0.001, DVAMS happiness B= 1.018 (0.858-1.178), *β*= 0.462, *p*<0.001, DVAMS alertness B= 0.907 (0.746-1.067), *β*= 0.419, *p*<0.001). Trait positive affect (PANAS-SF positive) was shown to be significantly negatively associated with participant pre-intervention SPANE negative scores (B= -0.105 (-0.132- -0.079), *β*= -0.308, *p*<0.001).

Trait negative affect (PANAS-SF negative) was shown to be an independent predictor of all pre-intervention measures, whereby high trait negative affect scores predicted lower scores for the majority of positive mood measures (SPANE positive B= -0.345 (-0.406- -0.283), *β*= -0.414, *p*<0.001, Affective Slider happiness B= -0.948 (-1.120- -0.850), *β*= -0.428, *p*<0.001, Affective Slider alertness B= -0.968 (-1.205- -0.730), *β*= -0.312, *p*<0.001, DVAMS happiness B= -1.395 (-1.607- -1.183), *β*= -0.474, *p*<0.001, DVAMS alertness B= -0.894 (-1.114- -0.673), *β*= -0.312, *p*<0.001). The exception was the SPANE negative (B= 0.292 (0.262-0.322), *β*= 0.617, *p*<0.001) that resulted in higher scores in participants with high trait negative affect.

**Table 5.11**: Multiple regression analyses of perceived stress and trait affect predicting pre-intervention SPANE positive scores.

| | Regression 1 | Regression 2 | Regression 3 | Regression 4 |
|---|---|---|---|---|
| **Age (per year)** | -0.066 | -0.059 | -0.041 | **-0.084*** |
| **Sex (female)** | 0.020 | **0.104**** | 0.032 | 0.054 |
| **Perceived Stress** | - | **-0.487***** | | |
| **PANAS-SF Positive** | - | - | **0.512***** | |
| **PANAS-SF Negative** | - | - | | **-0.414***** |
| **R** | 0.069 | 0.484 | 0.516 | 0.417 |
| **$R^2$** | 0.005 | 0.234 | 0.266 | 0.174 |

*β* values are reported in the table. *$p$<.05, **$p$<.01, ***$p$<.001.

**Table 5.12**: Multiple regression analyses of perceived stress and trait affect predicting pre-intervention SPANE negative scores.

| | Regression 1 | Regression 2 | Regression 3 | Regression 4 |
|---|---|---|---|---|
| **Age (per year)** | -0.018 | -0.020 | -0.012 | 0.043 |
| **Sex (female)** | 0.030 | **-0.074*** | 0.054 | 0.007 |
| **Perceived Stress** | | **0.539***** | | |
| **PANAS-SF Positive** | | | **-0.308***** | |
| **PANAS-SF Negative** | | | | **0.617***** |
| **R** | 0.035 | 0.532 | 0.312 | 0.616 |
| **$R^2$** | 0.001 | 0.283 | 0.097 | 0.380 |

*β* values are reported in the table. *$p$<.05, **$p$<.01, ***$p$<.001.

**Table 5.13**: Multiple regression analyses of perceived stress and trait affect predicting pre-intervention Affective Slider happiness scores.

| | Regression 1 | Regression 2 | Regression 3 | Regression 4 |
|---|---|---|---|---|
| **Age (per year)** | -0.006 | -0.005 | 0.017 | -0.022 |
| **Sex (female)** | -0.037 | 0.049 | -0.022 | 0.007 |
| **Perceived Stress** | | **-0.476***** | | |
| **PANAS-SF Positive** | | | **0.414***** | |
| **PANAS-SF Negative** | | | | **-0.428***** |
| **R** | 0.037 | 0.470 | 0.415 | 0.428 |
| **$R^2$** | 0.001 | 0.220 | 0.172 | 0.183 |

*β* values are reported in the table. *$p$<.05, **$p$<.01, ***$p$<.001.

**Table 5.14**: Multiple regression analyses of perceived stress and trait affect predicting pre-intervention Affective Slider alertness scores.

| | Regression 1 | Regression 2 | Regression 3 | Regression 4 |
|---|---|---|---|---|
| **Age (per year)** | 0.003 | -0.001 | 0.026 | -0.022 |
| **Sex (female)** | -0.069 | 0.005 | -0.041 | -0.054 |
| **Perceived Stress** | | **-0.392*** ** | | |
| **PANAS-SF Positive** | | | **0.437*** ** | |
| **PANAS-SF Negative** | | | | **-0.312*** ** |
| **R** | 0.069 | 0.391 | 0.439 | 0.320 |
| **$R^2$** | 0.005 | 0.153 | 0.193 | 0.102 |

*β* values are reported in the table. *$p$<.05, **$p$<.01, ***$p$<.001.

**Table 5.15:** Multiple regression analyses of perceived stress and trait affect predicting pre-intervention DVAMS happiness scores.

| | Regression 1 | Regression 2 | Regression 3 | Regression 4 |
|---|---|---|---|---|
| **Age (per year)** | -0.035 | -0.040 | -0.024 | -0.063 |
| **Sex (female)** | 0.038 | **0.127*** ** | 0.038 | 0.071 |
| **Perceived Stress** | | **-0.479*** ** | | |
| **PANAS-SF Positive** | | | **0.462*** ** | |
| **PANAS-SF Negative** | | | | **-0.474** |
| **R** | 0.052 | 0.475 | 0.465 | 0.474 |
| **$R^2$** | 0.003 | 0.226 | 0.216 | 0.225 |

*β* values are reported in the table. *$p$<.05, **$p$<.01, ***$p$<.001.

**Table 5.16:** Multiple regression analyses of perceived stress and trait affect predicting pre-intervention DVAMS alertness scores.

| | Regression 1 | Regression 2 | Regression 3 | Regression 4 |
|---|---|---|---|---|
| **Age (per year)** | -0.046 | -0.054 | -0.038 | -0.065 |
| **Sex (female)** | -0.086 | -0.021 | -0.073 | **-0.077*** |
| **Perceived Stress** | | **-0.383*** ** | | |
| **PANAS-SF Positive** | | | **0.419*** ** | |
| **PANAS-SF Negative** | | | | **-0.312*** ** |
| **R** | 0.098 | 0.390 | 0.428 | 0.328 |
| **$R^2$** | 0.010 | 0.152 | 0.183 | 0.108 |

*β* values are reported in the table. *$p$<.05, **$p$<.01, ***$p$<.001.

#### *5.5.1.2 To what extent did psychological factors at baseline influence post-intervention positive mood on the day of vaccination?*

##### 5.5.1.2.1 Hypothesis

Unsurprisingly, the previous analyses suggested that baseline psychological factors (perceived stress and positive and negative trait affect) were predictors of pre-intervention state affect. However, more importantly, it was essential to determine whether these baseline psychological factors played a role in influencing how well the interventions worked at increasing positive mood. For participants with high levels of negative trait affect and perceived stress and/or low levels of positive trait affect, it was hypothesised that allocation to an intervention group would result in greater improvements in positive state affect due to there being larger room for improvement compared to participants with lower perceived stress and negative trait affect.

##### 5.5.1.2.2 Analyses

Multiple regression analyses were carried out to investigate whether perceived stress (PSS scale) and trait mood (PANAS-SF positive and PANAS-SF negative) measured at baseline were significant independent predictors of post-intervention state mood (SPANE positive, SPANE negative, Affective Slider happiness, Affective Slider alertness, DVAMS happiness and DVAMS alertness). In contrast to the above analyses, these analyses were conducted separately for each arm of the For-ME trial as opposed to analyses of the whole cohort. This is because the results in *Section 5.4.1* have shown that allocation to an intervention group resulted in greater levels of post-intervention positive state affect compared with usual care.

Due to the significant associations between the baseline measures (perceived stress and PANAS-SF positive and negative), outlined in *Section 5.3.3.1*, separate two-step regression models were run for each baseline variable (i.e., Step 1: age, sex and pre-intervention score for each state affect outcome measure, Step 2: perceived stress/ PANAS-SF positive/ PANAS-SF negative). A total of 4 regression analyses were run for each trial arm for the following: *Regression 1*= age, sex and pre-intervention score, *Regression 2*= perceived stress, *Regression 3*= positive trait affect, *Regression 4*= negative trait affect (see *Table 5.17- 5.22).*

**Table 5.17:** Multiple regression analyses of perceived stress and trait affect predicting post-intervention Affective Slider happiness scores for the usual care group.

| | Regression 1 | Regression 2 | Regression 3 | Regression 4 |
|---|---|---|---|---|
| **Age (per year)** | 0.050 | 0.076 | 0.052 | 0.083 |
| **Sex (female)** | 0.045 | 0.036 | 0.035 | 0.045 |
| **Pre-Intervention Score** | **0.873*** ** | **0.819*** ** | **0.777*** ** | **0.859*** ** |
| **Perceived Stress** | | -0.061 | | |
| **PANAS-SF Positive** | | | 0.147 | |
| **PANAS-SF Negative** | | | | -0.009 |
| **R** | 0.866 | 0.845 | 0.841 | 0.855 |
| **$R^2$** | 0.749 | 0.715 | 0.707 | 0.731 |

*β* values are reported in the table. **p*<.05, ***p*<.01, ****p*<.001.

**Table 5.18**: Multiple regression analyses of perceived stress and trait affect predicting post-intervention Affective Slider happiness scores for the standardised intervention group.

| | Regression 1 | Regression 2 | Regression 3 | Regression 4 |
|---|---|---|---|---|
| **Age (per year)** | -0.095 | -0.099 | -0.071 | -0.018 |
| **Sex (female)** | 0.038 | **0.039*** | 0.041 | 0.027 |
| **Pre-Intervention Score** | **0.694*** ** | **0.682*** ** | **0.677*** ** | **0.767*** ** |
| **Perceived Stress** | | -0.024 | | |
| **PANAS-SF Positive** | | | 0.049 | |
| **PANAS-SF Negative** | | | | 0.081 |
| **R** | 0.701 | 0.689 | 0.707 | 0.737 |
| **$R^2$** | 0.491 | 0.474 | 0.500 | 0.543 |

*β* values are reported in the table. **p*<.05, ***p*<.01, ****p*<.001.

**Table 5.19:** Multiple regression analyses of perceived stress and trait affect predicting post-intervention Affective Slider happiness scores for the choice intervention group.

| | Regression 1 | Regression 2 | Regression 3 | Regression 4 |
|---|---|---|---|---|
| **Age (per year)** | -0.117 | **-0.118*** | **-0.109*** | -0.125 |
| **Sex (female)** | 0.115 | **0.131** ** | **0.117*** | 0.110 |
| **Pre-Intervention Score** | **0.641*** ** | **0.605*** ** | **0.584*** ** | **0.607*** ** |
| **Perceived Stress** | | -0.078 | | |
| **PANAS-SF Positive** | | | 0.133 | |
| **PANAS-SF Negative** | | | | -0.060 |
| **R** | 0.657 | 0.660 | 0.660 | 0.649 |
| **$R^2$** | 0.432 | 0.436 | 0.436 | 0.421 |

*β* values are reported in the table. **p*<.05, ***p*<.01, ****p*<.001.

**Table 5.20:** Multiple regression analyses of perceived stress and trait affect predicting post-intervention Affective Slider alertness scores for the usual care group.

| | Regression 1 | Regression 2 | Regression 3 | Regression 4 |
|---|---|---|---|---|
| **Age (per year)** | -0.003 | -0.013 | -0.023 | -0.021 |
| **Sex (female)** | -0.034 | -0.049 | -0.024 | -0.039 |
| **Pre-Intervention Score** | **0.888*** ** | **0.876*** ** | **0.879*** ** | **0.916*** ** |
| **Perceived Stress** | | 0.023 | | |
| **PANAS-SF Positive** | | | 0.044 | |
| **PANAS-SF Negative** | | | | 0.072 |
| **R** | 0.892 | 0.872 | 0.900 | 0.895 |
| **$R^2$** | 0.795 | 0.761 | 0.809 | 0.800 |

*β* values are reported in the table. *$p$<.05, **$p$<.01, ***$p$<.001.

**Table 5.21:** Multiple regression analyses of perceived stress and trait affect predicting post-intervention Affective Slider alertness scores for the standardised intervention group.

| | Regression 1 | Regression 2 | Regression 3 | Regression 4 |
|---|---|---|---|---|
| **Age (per year)** | -0.020 | -0.015 | 0.020 | -0.003 |
| **Sex (female)** | **0.094*** | **0.109**** | **0.100*** | **0.086*** |
| **Pre-Intervention Score** | **0.769*** ** | **0.811*** ** | **0.775*** ** | **0.773*** ** |
| **Perceived Stress** | | -0.013 | | |
| **PANAS-SF Positive** | | | 0.038 | |
| **PANAS-SF Negative** | | | | -0.068 |
| **R** | 0.767 | 0.816 | 0.796 | 0.792 |
| **$R^2$** | 0.588 | 0.666 | 0.634 | 0.627 |

*β* values are reported in the table. *$p$<.05, **$p$<.01, ***$p$<.001.

**Table 5.22:** Multiple regression analyses of perceived stress and trait affect predicting post-intervention Affective Slider alertness scores for the choice intervention group.

| | Regression 1 | Regression 2 | Regression 3 | Regression 4 |
|---|---|---|---|---|
| **Age (per year)** | -0.061 | **-0.082*** | **-0.091*** | -0.073 |
| **Sex (female)** | 0.065 | 0.064 | 0.054 | 0.058 |
| **Pre-Intervention Score** | **0.747*** ** | **0.740*** ** | **0.733*** ** | **0.734*** ** |
| **Perceived Stress** | | -0.039 | | |
| **PANAS-SF Positive** | | | 0.036 | |
| **PANAS-SF Negative** | | | | -0.028 |
| **R** | 0.752 | 0.762 | 0.756 | 0.749 |
| **$R^2$** | 0.565 | 0.581 | 0.572 | 0.561 |

*β* values are reported in the table. *$p$<.05, **$p$<.01, ***$p$<.001.

#### 5.5.1.2.3 Results

Analyses indicated that participants with high levels of perceived stress and negative trait affect, measured at baseline, scored lower pre-intervention positive state affect and higher negative state affect (SPANE negative). Additionally, participants with high positive trait affect at baseline scored higher pre-intervention state affect scores for all measures except for the SPANE negative scale. However, contrary to what was stipulated in the hypothesis, perceived stress and trait affect measured at baseline were not found to be predictors of post-intervention state affect. Results for this are outlined in *Tables 5.17- 5.22* for the Affective Slider happiness and alertness subscales. However, similar results were observed for all positive state affect outcome measures.

### *5.5.2 Exploratory Question 2: To assess whether psychological factors measured at baseline were associated with antibody responses to influenza vaccination.*

#### *5.5.2.1 Do psychological factors measured at baseline predict antibody responses to influenza vaccination?*

##### 5.5.2.1.1 Hypotheses

Previous research (outlined in *Chapter 2*) has provided a plethora of evidence indicating the immunosuppressive effects of stress, specifically chronically stressed older adults who have been shown to present lower antibody responses to vaccination (Burns et al., 2003; Glaser et al., 1987, 1998, 2000; Pedersen et al., 2009; Phillips et al., 2005; Segerstrom et al., 2008, 2012; Vedhara et al., 1999). It was therefore hypothesised that participants with higher levels of perceived stress measured at baseline would present lower 4-weeks post-vaccination antibody responses. Additionally, trait negative affect (e.g., depression) and trait positive affect (e.g., dispositional optimism, wellbeing) have been outlined (see *Chapter 2*) to modulate immune responses to vaccination (Herbert & Cohen, 1993; Marsland et al., 2006). Similar to stress, negative affect such as depression has been indicated to downregulate antibody responses to vaccination (Afsar et al., 2009). It was therefore hypothesised that participants with higher levels of baseline trait negative affect would present lower 4-weeks post-vaccination antibody responses. On the contrary, trait positive affect has been indicated to have immune enhancing effects (Marsland et al., 2006, 2007), where it was also hypothesised that participants with higher levels of trait positive affect measured at baseline would present higher 4-weeks post-vaccination antibody responses. Finally, there has also been evidence outlining the influence of age and sex on vaccination responses (Boraschi & Italiani, 2014; Giefing-Kröll et al., 2015), whereby it was also hypothesised that these factors could be possible predictors of antibody responses to vaccination, with increasing age being associated with lower 4-weeks post-vaccination responses and no directional hypothesis for gender due to mixed findings in previous research.

##### 5.5.2.1.2 Analyses

Analyses were conducted to investigate whether participant baseline perceived stress and trait affect (PANAS-SF negative and positive) were predictors of 4-weeks post- vaccination antibody responses for all vaccine components (A/Brisbane/02/2018 (H1N1), A/Kansas/14/2017 (H3N2), B/Colorado/06/2017 (B)). Similar to the previous section assessing antibody responses to vaccination, this section presents both ELISA and HAI analyses.

#### 5.5.2.1.2.1 Enzyme- Linked Immunosorbent Assay

Multiple regression analyses were carried out to investigate whether 4-weeks post-vaccination ELISA OD levels were predicted by perceived stress and trait affect (see *Table 5.23, Table 5.24 and Table 5.25*). Similar to the previous exploratory analyses, age and sex were controlled for prior to the addition of other variables to the regression models (*Regression 1* highlighted in the tables). Neither of these confounding factors were found to be predictors of 4-week post-vaccination antibody responses for any of the vaccine strains in terms of ELISA OD levels. There was no statistical significance in these analyses that suggested perceived stress or trait affect measured at baseline were predictors of 4-weeks post-vaccination antibody responses.

#### 5.5.2.1.2.2 Hemagglutinin Inhibition Assay

Binary logistic regression analyses were carried out to investigate whether perceived stress and trait affect predicted whether participants were classified as seroprotected for HAI thresholds ≥40 and ≥160 (see *Tables 5.26- 5.31)*. Age and sex were selected as covariates as part of these regression models to determine if they were independent predictors of antibody responses to vaccination. Similar to the results presented above, neither age or sex were found to be predictors of seroprotection for either threshold for 2 out of 3 of the vaccine components. Age was found to be a statistically significant predictor of seroprotection (≥40 and ≥160) for influenza strain A/Brisbane/02/2018 (H1N1) before baseline variables of perceived stress and trait affect were considered (see *Table 5.26 and Table 5.29*. A/Brisbane/02/2018 (H1N1) for ≥40 B= -0.113, Odds Ratio= 0.875 (0.829-0.924) and ≥160 B= -0.095, Odds Ratio= 0.909 (0.880-0939)), suggesting that older participants were less likely to achieve seroprotection thresholds post-vaccination. This highlighted the importance of computing separate regression models for these confounding factors, and for each baseline variable of interest that have been shown to be significantly correlated (see *Section 5.3.3.1*). These data indicate that, prior to taking into account baseline variables and after accounting for sex, participants were 11.3% and 9.5% less likely to be classified as seroprotected for influenza strain A/Brisbane/02/2018 (H1N1) for thresholds ≥40 and ≥160, respectively. After taking into account age and sex, these analyses showed no statistically significant associations between perceived stress or trait affect, and seroprotection classification.

**Table 5.23**: Multiple regression analyses of perceived stress and trait affect predicting post-vaccination A/Brisbane/02/2018 (H1N1) IgG levels.

| | Regression 1 | Regression 2 | Regression 3 | Regression 4 |
|---|---|---|---|---|
| **Age (per year)** | -0.068 | -0.061 | **-0.090*** | -0.060 |
| **Sex (female)** | 0.031 | 0.037 | 0.048 | 0.045 |
| **Perceived Stress** | | 0.047 | | |
| **PANAS-SF Positive** | | | **-0.098*** | |
| **PANAS-SF Negative** | | | | 0.081 |
| **R** | 0.074 | 0.091 | 0.141 | 0.117 |
| **$R^2$** | 0.006 | 0.008 | 0.020 | 0.014 |

*β* values are reported in the table. *$p$<.05, **$p$<.01, ***$p$<.001.

**Table 5.24:** Multiple regression analyses of perceived stress and trait affect predicting post-vaccination A/Kansas/14/2017 (H3N2) IgG levels.

| | Regression 1 | Regression 2 | Regression 3 | Regression 4 |
|---|---|---|---|---|
| **Age (per year)** | -0.071 | -0.060 | -0.083 | -0.060 |
| **Sex (female)** | 0.040 | 0.045 | 0.045 | 0.060 |
| **Perceived Stress** | | -0.014 | | |
| **PANAS-SF Positive** | | | -0.063 | |
| **PANAS-SF Negative** | | | | 0.006 |
| **R** | 0.082 | 0.075 | 0.113 | 0.086 |
| **$R^2$** | 0.007 | 0.006 | 0.013 | 0.007 |

*β* values are reported in the table. *$p$<.05, **$p$<.01, ***$p$<.001.

**Table 5.25:** Multiple regression analyses of perceived stress and trait affect predicting post-vaccination B/Colorado/06/2017 (B) IgG levels.

| | Regression 1 | Regression 2 | Regression 3 | Regression 4 |
|---|---|---|---|---|
| **Age (per year)** | 0.080 | 0.068 | 0.076 | 0.077 |
| **Sex (female)** | -0.010 | -0.006 | 0.007 | 0.010 |
| **Perceived Stress** | | 0.056 | | |
| **PANAS-SF Positive** | | | **-0.091*** | |
| **PANAS-SF Negative** | | | | 0.071 |
| **R** | 0.080 | 0.087 | 0.120 | -0.101 |
| **$R^2$** | 0.006 | 0.008 | 0.014 | 0.010 |

*β* values are reported in the table. *$p$<.05, **$p$<.01, ***$p$<.001.

**Table 5.26**: Binary logistic regression analyses of perceived stress and trait affect predicting post-vaccination A/Brisbane/02/2018 (H1N1) HAI seroprotection (threshold ≥40).

| | Regression 1 | Regression 2 | Regression 3 | Regression 4 |
|---|---|---|---|---|
| **Age (per year)** | **0.870*** ** | **0.875*** ** | **0.872*** ** | **0.875*** ** |
| **Sex (female)** | 0.745 | 0.657 | 0.614 | 0.795 |
| **Perceived Stress** | | 0.971 | | |
| **PANAS-SF Positive** | | | 1.021 | |
| **PANAS-SF Negative** | | | | 0.990 |
| **$R^2$ (Nagelkerke)** | 0.098 | 0.091 | 0.102 | 0.086 |

Odds ratio (Expected B) values are reported in the table. *$p$<.05, **$p$<.01, ***$p$<.001.

**Table 5.27:** Binary logistic regression analyses of perceived stress and trait affect predicting post-vaccination A/Kansas/14/2017 (H3N2) HAI seroprotection (threshold ≥40).

| | Regression 1 | Regression 2 | Regression 3 | Regression 4 |
|---|---|---|---|---|
| **Age (per year)** | 1.021 | 1.034 | 1.024 | 1.039 |
| **Sex (female)** | 0.720 | 0.710 | 0.683 | 0.729 |
| **Perceived Stress** | | 0.993 | | |
| **PANAS-SF Positive** | | | 0.999 | |
| **PANAS-SF Negative** | | | | 0.997 |
| **$R^2$ (Nagelkerke)** | 0.007 | 0.011 | 0.010 | 0.012 |

Odds ratio (Expected B) values are reported in the table. *$p$<.05, **$p$<.01, ***$p$<.001.

**Table 5.28:** Binary logistic regression analyses of perceived stress and trait affect predicting post-vaccination B/Colorado/06/2017 (B) HAI seroprotection (threshold ≥40).

| | Regression 1 | Regression 2 | Regression 3 | Regression 4 |
|---|---|---|---|---|
| **Age (per year)** | 1.046 | 1.040 | 1.040 | 1.046 |
| **Sex (female)** | 1.158 | 1.193 | 1.154 | 1.219 |
| **Perceived Stress** | | 1.035 | | |
| **PANAS-SF Positive** | | | 0.980 | |
| **PANAS-SF Negative** | | | | 1.041 |
| **$R^2$ (Nagelkerke)** | 0.007 | 0.013 | 0.009 | 0.013 |

Odds ratio (Expected B) values are reported in the table. *$p$<.05, **$p$<.01, ***$p$<.001.

**Table 5.29**: Binary logistic regression analyses of perceived stress and trait affect predicting post-vaccination A/Brisbane/02/2018 (H1N1) HAI seroprotection (threshold ≥160).

| | Regression 1 | Regression 2 | Regression 3 | Regression 4 |
|---|---|---|---|---|
| **Age (per year)** | **0.909*** ** | **0.913*** ** | **0.903*** ** | **0.908*** ** |
| **Sex (female)** | 0.789 | 0.772 | 0.684 | 0.807 |
| **Perceived Stress** | | 1.011 | | |
| **PANAS-SF Positive** | | | 0.984 | |
| **PANAS-SF Negative** | | | | 1.026 |
| **$R^2$ (Nagelkerke)** | 0.074 | 0.071 | 0.091 | 0.082 |

Odds ratio (Expected B) values are reported in the table. *$p$<.05, **$p$<.01, ***$p$<.001.

**Table 5.30**: Binary logistic regression analyses of perceived stress and trait affect predicting post-vaccination A/Kansas/14/2017 (H3N2) HAI seroprotection (threshold ≥160).

| | Regression 1 | Regression 2 | Regression 3 | Regression 4 |
|---|---|---|---|---|
| **Age (per year)** | 0.973 | 0.983 | 0.977 | 0.978 |
| **Sex (female)** | 0.780 | **0.697*** | 0.727 | 0.752 |
| **Perceived Stress** | | **0.975*** | | |
| **PANAS-SF Positive** | | | 1.004 | |
| **PANAS-SF Negative** | | | | 0.978 |
| **$R^2$ (Nagelkerke)** | 0.011 | 0.018 | 0.013 | 0.014 |

Odds ratio (Expected B) values are reported in the table. *$p$<.05, **$p$<.01, ***$p$<.001.

**Table 5.31:** Binary logistic regression analyses of perceived stress and trait affect predicting post-vaccination B/Colorado/06/2017 (B) HAI seroprotection (threshold ≥160).

| | Regression 1 | Regression 2 | Regression 3 | Regression 4 |
|---|---|---|---|---|
| **Age (per year)** | 1.004 | 0.999 | 1.012 | 1.006 |
| **Sex (female)** | 1.137 | 1.166 | 1.082 | 1.108 |
| **Perceived Stress** | | 1.015 | | |
| **PANAS-SF Positive** | | | 0.980 | |
| **PANAS-SF Negative** | | | | 1.014 |
| **$R^2$ (Nagelkerke)** | 0.001 | 0.005 | 0.009 | 0.003 |

Odds ratio (Expected B) values are reported in the table. *$p$<.05, **$p$<.01, ***$p$<.001.

#### 5.5.2.1.3 Summary of Results

In contrast to the hypothesis, there was very limited evidence within this sample that indicated that age or sex meaningfully influenced influenza vaccination responses. This limited evidence suggested that with each yearly increase in age, participants were 11.3% and 9.5% less likely to be classified as seroprotected for influenza strain A/Brisbane/02/2018 (H1N1) for thresholds ≥40 and ≥160, respectively. However, age was not shown to be a predictor for seroprotection of influenza strains A/Kansas/14/2017 (H3N2) or B/Colorado/06/2017 (B), as well as ELISA OD levels for any vaccine components. These analyses also indicated that perceived stress and trait affect measured at baseline were not significantly associated with 4-week post-vaccination antibody responses in terms of ELISA OD levels or in terms of the proportion of participants classified as seroprotected for either threshold.

### *5.5.3 Exploratory Question 3: To compare two different methods of measuring antibody responses to influenza vaccination.*

#### *5.5.3.1 Were these two methods of measuring antibody responses to vaccination correlated? Were the proportion of participants who meet the ELISA OD equivalents of HAI seroprotection thresholds similar to those classified as seroprotected? Are ELISA and HAI interchangeable methods of measuring influenza vaccination responses?*

##### 5.5.3.1.1 Hypothesis

ELISA and HAI are two different methods of measuring antibody responses to vaccination. ELISA allows for greater differentiation between samples due to this assay being a continuous outcome measure, and has the ability to measure different class-specific antibodies (IgM, IgA and IgG) in serum samples in response to vaccination. It is particularly useful for large-scale analyses because it can be completed within a few hours and is amenable to complete automation. For this reason, ELISA is considered to be a sensitive, flexible and low-cost laboratory technique readily used to measure antibody responses to vaccination (Coudeville et al., 2010). However, HAI is considered to be the gold standard for assessing vaccination responses. The HAI assay relies on the ability of hemagglutinin-specific antibodies (predominantly IgG) to inhibit the binding between the hemagglutinin of the virus and the sialic acid receptors on the surface of red blood cells (from chicken or turkey mainly). The nature of this assay means that there is no gradation between assay serial dilutions making it difficult to distinguish between samples, therefore making it a less sensitive method compared with ELISA. The HAI assay has a well-established clinical protection threshold of ≥40 cited in the influenza vaccination literature (Kirchenbaum et al., 2021). However, this threshold is being challenged due to it not being applicable to all virus types, such as influenza, whereby older adult populations are subject to extensive previous exposure (naturally and/or via previous vaccination). More recently higher HAI titre thresholds are being considered, such as ≥160, to better identify protection for older adult populations (Zhao et al., 2017).

Whilst these methods of measuring antibody responses to vaccination differ, it was hypothesised that they would be significantly correlated with each other. Analyses also investigated whether it was possible to determine ELISA OD values equivalent to HAI geometric mean values to examine whether the same proportion of participants were classified as seroprotected using these two different methods. These methods were also

compared by assessing their level of agreement to determine whether they could be interchangeable methods of measuring antibody responses to influenza vaccination.

#### 5.5.3.1.2 Analyses

Bivariate correlations (Pearson's) were computed to assess the relationship between the HAI geometric mean and ELISA OD values for each influenza strain of the vaccine (see *Table 5.32*). As expected, these analyses indicated that the outcome measures of these methods of measuring antibody responses to vaccination were significantly correlated with each other when measuring the same influenza strain (H1N1: r= 0.590, $p$<0.010, H3N2: r= 0.698, $p$<0.010 and B: r= 0.577, $p$<0.010).

**Table 5.32:** Bivariate correlations (Pearson's) between ELISA OD levels and HAI geometric mean measures for H1N1, H3N2 and B influenza vaccine strains.

| | **1** | **2** | **3** | **4** | **5** | **6** |
|---|---|---|---|---|---|---|
| **(1) ELISA OD A/Brisbane/02/2018 (H1N1)** | - | - | - | **0.590**** | - | - |
| **(2) ELISA OD A/Kansas/14/2017 (H3N2)** | - | - | - | - | **0.698**** | - |
| **(3) ELISA OD B/Colorado/06/2017-like (B)** | - | - | - | - | - | **0.577**** |
| **(4) HAI Geometric Mean A/Brisbane/02/2018 (H1N1)** | **0.590**** | - | - | - | - | - |
| **(5) HAI Geometric Mean A/Kansas/14/2017 (H3N2)** | - | **0.698**** | - | - | - | - |
| **(6) HAI Geometric Mean B/Colorado/06/2017 (B)** | - | - | **0.577**** | - | - | - |

*$p$<.05, **$p$<.01, ***$p$<.001

Significant positive correlations between these two different methods of measuring antibody responses to vaccination are shown graphically in *Figure 5.9*. These graphs indicate a line of best fit for each influenza strain that has been used to determine the ELISA OD equivalents of both seroprotection thresholds. These analyses were done to investigate whether the same proportion of participants met these seroprotection thresholds for HAI and ELISA measures. Firstly, log2 geometric mean values for HAI thresholds ≥40 and ≥160 were determined and were found to be 5.322 and 7.322, respectively. These values were then

input into the line of best fit equations for each influenza strain to interpolate the equivalent ELISA OD values associated with these HAI thresholds. These analyses found that a ≥40 HAI titre threshold equated to ELISA OD values of 0.858, 0.811 and 1.078 for strains H1N1, H3N2 and B, respectively. Additionally, a ≥160 HAI threshold equated to ELISA OD values of 1.056, 1.041 and 1.383 for strains H1N1, H3N2 and B, respectively. These values fall on the lower end of the OD scale (>1 to 3) for this ELISA essay. Once these ELISA OD values were calculated, the proportion of participants who met this threshold were computed to compare with the HAI data. In *Table 5.33*, a summary of the percentage of participants who were classified as HAI seroprotected for thresholds titres ≥40 and ≥160 are outlined along with the ELISA OD equivalent values of these thresholds. The percentage of participants with ≥40 HAI titre thresholds were much higher for both pre- and post-vaccination across all influenza strains compared with ELISA OD equivalent percentages. However, the percentage of participants with ≥160 HAI titre thresholds were more similar to the percentages of participants with the ELISA OD equivalent percentages, with HAI percentages slightly higher than ELISA OD percentages for pre- and post-vaccination. This suggests that whilst there is large variability between the lower threshold data, the smaller variability of the higher threshold data may indicate there is a chance of there being some agreement between these two methods of measuring antibody responses but requires further analyses.

**Table 5.33**: Percentage of participants classified as being seroprotected (4-weeks post-vaccination) (for HAI thresholds ≥40 and ≥160) compared with ELISA OD equivalents of these HAI thresholds.

| | Frequency (%) |
|---|---|
| **Seroprotection (titre ≥40)** | |
| A/Brisbane/02/2018 (H1N1) (Pre) | 406 (63.7%) |
| A/Brisbane/02/2018 (H1N1) (Post) | 541 (89.7%) |
| A/Kansas/14/2017 (H3N2) (Pre) | 188 (29.5%) |
| A/Kansas/14/2017 (H3N2) (Post) | 533 (88.4%) |
| B/Colorado/06/2017 (B) (Pre) | 509 (79.9%) |
| B/Colorado/06/2017 (B) (Post) | 577 (95.7%) |
| **ELISA OD Equivalent of HAI ≥40** | |
| A/Brisbane/02/2018 (H1N1) (Pre) | 171 (26.3%) |
| A/Brisbane/02/2018 (H1N1) (Post) | 311 (52.9%) |
| A/Kansas/14/2017 (H3N2) (Pre) | 221 (34.0%) |
| A/Kansas/14/2017 (H3N2) (Post) | 446 (75.9%) |
| B/Colorado/06/2017-like (B) (Pre) | 398 (61.0%) |
| B/Colorado/06/2017-like (B) (Post) | 489 (83.2%) |
| **Seroprotection (titre ≥160)** | |
| A/Brisbane/02/2018 (H1N1) (Pre) | 190 (29.1%) |
| A/Brisbane/02/2018 (H1N1) (Post) | 416 (63.6%) |
| A/Kansas/14/2017 (H3N2) (Pre) | 76 (11.6%) |
| A/Kansas/14/2017 (H3N2) (Post) | 436 (66.7%) |
| B/Colorado/06/2017 (B) (Pre) | 286 (43.7%) |
| B/Colorado/06/2017 (B) (Post) | 439 (67.1%) |
| **ELISA OD Equivalent of HAI ≥160** | |
| A/Brisbane/02/2018 (H1N1) (Pre) | 182 (28.0%) |
| A/Brisbane/02/2018 (H1N1) (Post) | 327 (55.6%) |
| A/Kansas/14/2017 (H3N2) (Pre) | 110 (16.9%) |
| A/Kansas/14/2017 (H3N2) (Post) | 325 (55.3%) |
| B/Colorado/06/2017-like (B) (Pre) | 255 (39.2%) |
| B/Colorado/06/2017-like (B) (Post) | 370 (62.9%) |

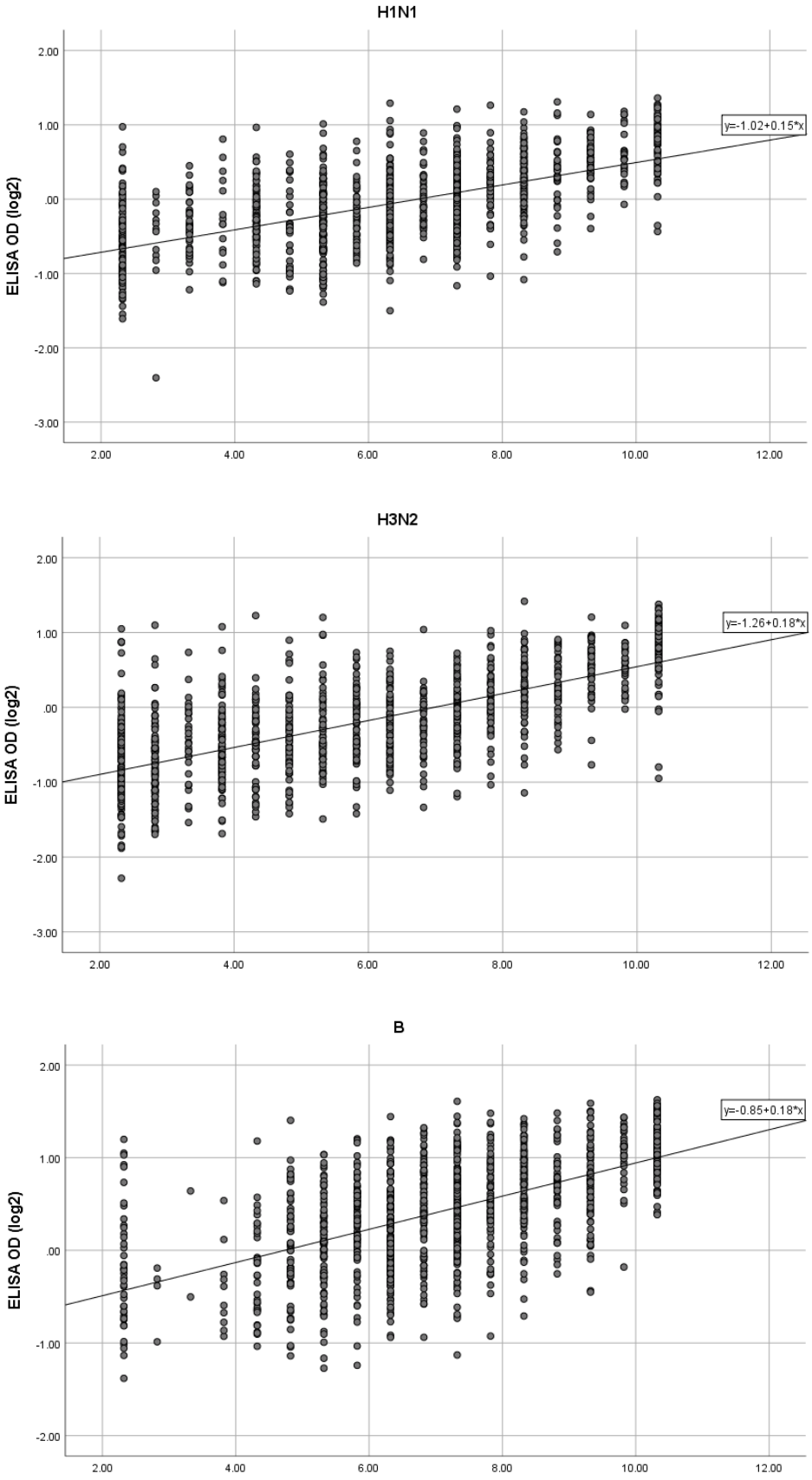



**Figure 5.9**: Relationships between ELISA OD measures and HAI geometric mean measures for H1N1, H3N2 and B influenza vaccine strains, including pre- and post-vaccination measures.

Whilst these two methods of measuring antibody responses to vaccination were significantly correlated, this did not mean there was a good agreement between both methods and did not assess the comparison between them. In order to compare and assess the level of agreement between these two quantitative measures (ELISA and HAI), Bland-Altman analyses were conducted to determine whether ELISA OD levels and HAI geometric mean values have good agreement with each other (Bland & Altman, 1999). This statistical method is used to assess whether one technique for measuring a variable can substitute another. In this case, whilst both ELISA and HAI were highly correlated, it is unclear whether they could be used interchangeably for the detection of antibody responses to vaccination. The Bland-Altman method quantified agreement between two measurements by constructing limits of agreement that were calculated using the mean and the standard deviations of the differences between them. This was done for each separate vaccine component. These Bland-Altman plots are illustrated in *Figure 5.10,* whereby the difference of the two paired measurements is plotted against the mean of the two measurements. It is recommended that 95% of the data points should lie within ± 2 standard deviations of the mean difference. As illustrated in *Figure 5.10*, there were a large quantity of values outside (outlined in red) of the 95% confidence limits (indicated by the dotted lines) for all influenza strains. Regression analyses of these difference and mean values indicated that there is a considerable amount of proportional bias (H1N1: t= 49.843, $p<0.001$, H3N2: t= 52.866, $p<0.001$, B: t= 42.137, $p<0.001$), suggesting these methods of measuring antibody responses to vaccination were statistically different and therefore were not in agreement. This suggested that these methods of antibody measurement should not be considered interchangeable.

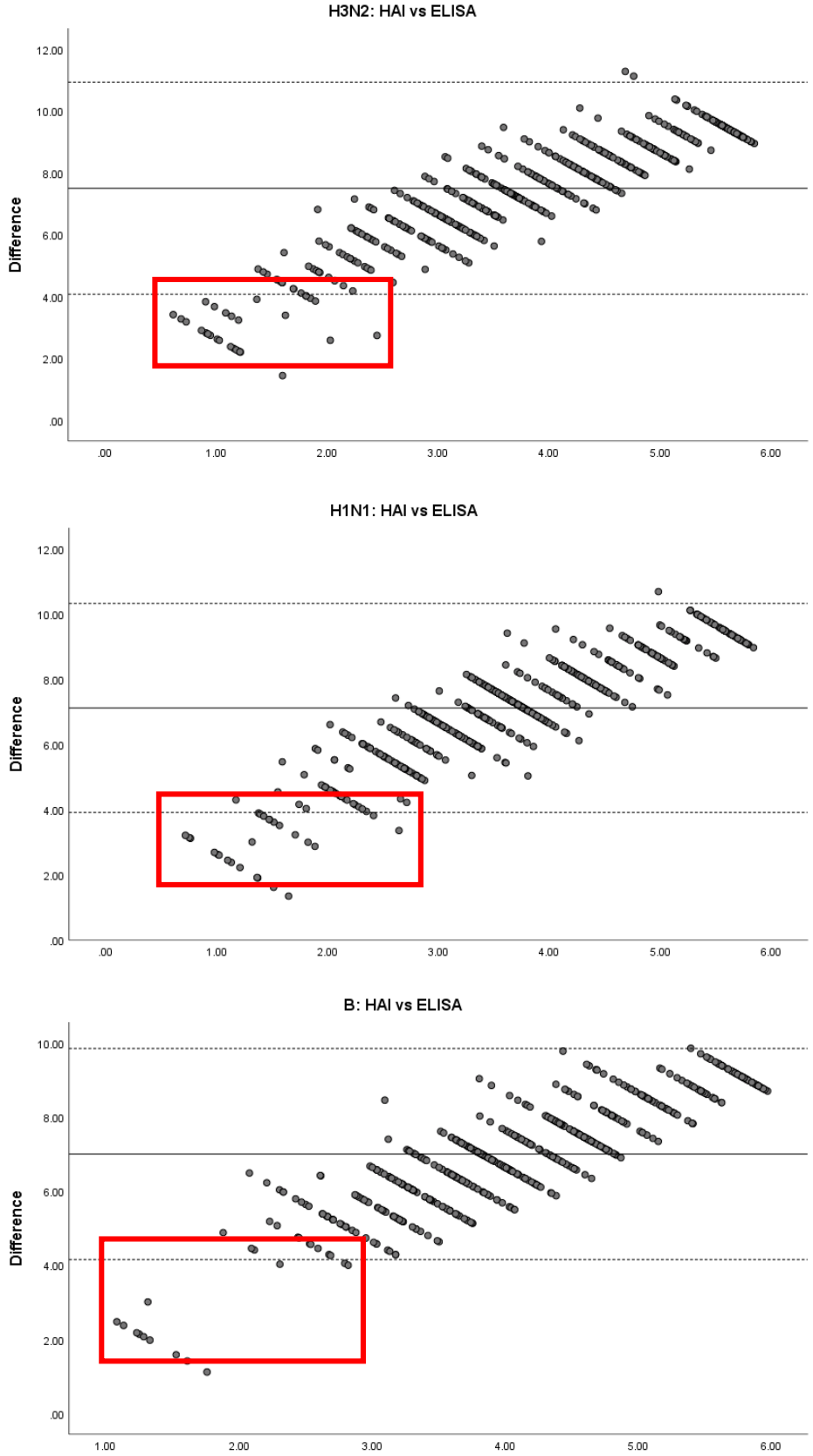



**Figure 5.10**: Bland-Altman agreement plots of antibody titres determined via HAI and ELISA for influenza vaccine components H1N1, H3N2 and B. Dotted lines indicate 95% confidence intervals.

#### 5.5.3.1.3 Results

These analyses indicated that ELISA and HAI were significantly correlated methods of measuring antibody responses to vaccination. ELISA OD values equivalent to HAI thresholds of ≥40 and ≥160 were determined, whereby the proportion of participants classified as seroprotected based on ELISA values were considerably lower for the ≥40 threshold compared with HAI, and were slightly lower for the ≥160 threshold compared with HAI. Despite some indication of agreement for the higher threshold, Blind-Altman analyses established that these two different methods of measuring antibody responses to vaccination were statistically significantly different, did not show comparable titres and therefore could not be utilised interchangeably.

## 5.6 Chapter Summary

These findings have shown that it is possible for a brief, 15-minute standardised and choice intervention administered within a clinical setting to significantly enhance the positive mood of older adults compared with usual care. Despite research suggesting choice interventions may be superior to those without choice, this trial did not show any statistical difference between these intervention types in terms of mood outcomes. Vaccination was shown to be effective, with statistically significant changes from pre- to post-vaccination antibody levels noted for all vaccine components measured by both ELISA and HAI. Group allocation to an intervention or usual care trial arm was not, however, a predictor of vaccination responses. In contrast to previous literature, neither perceived stress or trait affect predicted 4-week post-vaccination antibody responses. The two methods of measuring antibody responses to vaccination, ELISA and HAI, were highly correlated with each other but were not found to be in agreement or equivalent to be able to be used interchangeably. In the next chapter, the discussion of these results will be outlined in the context of similar literature, along with a general For-ME trial review.

# 6 Chapter 6: General Discussion

## 6.1 Introduction

This thesis has examined the potential to enhance positive mood at the time of vaccination in older adults, with a view to considering the implications for influenza vaccination effectiveness in this population. The emphasis within this thesis on influenza vaccination and older adults was driven by the large burden of infectious disease and poor vaccine efficacy observed for this vulnerable population (L. Feng et al., 2012; Govaert et al., 1994; Haq & McElhaney, 2014; NICE, 2019). In *Chapter 1*, the impact of infectious disease, and influenza specifically, on this population was discussed at length. In *Chapter 2,* the large and growing body of evidence that psychological factors can impact immunity, and in turn responses to vaccination was reviewed. The research conducted as part of this thesis contributes to, and extends, the evidence in this area.

Central to this thesis was the development, conduct, and analyses of a large multi-centre randomised controlled trial that examined the impact of two brief positive mood interventions administered prior to influenza vaccination (The For-ME trial). The rationale, trial design and methods for the For-ME trial were discussed in *Chapter 3*. To briefly reiterate this rationale, prior research had indicated that positive mood on the day of vaccination may be the most important psychological or behavioural predictor of antibody responses to influenza vaccination in older adults (Ayling et al., 2018). This longitudinal observational cohort study explored an array of behavioural (e.g., exercise, sleep and nutrition) and psychological (e.g., stress and mood) factors and their influence on influenza vaccination responses in older adults. Positive mood on the day of vaccination was found to be the most important predictor of vaccination responses, where it was associated with enhanced responses to influenza vaccination strain A(H1N1). Further pilot research then built on this finding, examining whether intervening to improve mood on the day of influenza vaccination in this population was feasible in primary care (Ayling et al., 2019). To do this, researchers developed a novel brief psychological intervention to improve positive mood and piloted it in primary care settings. This pilot trial found that participants who received this (fixed-content) intervention statistically improved positive mood (the positive mood outcome measure was the Affective Slider happiness subscale) compared with the neutral intervention. Further, the pilot trial found early suggestive evidence that such an approach may beneficially impact on antibody responses to vaccination with point-estimates for all

vaccine strains favouring the intervention arm compared to a neutral control arm (albeit this study was not powered to detect significant differences on this outcome).

The For-ME trial (described in *Chapters 3-5* of this thesis) was a much larger study (*n*=654), which builds upon this earlier research, with the primary aim of investigating whether the previously piloted brief standardised positive mood intervention and/or a novel intervention that incorporated participant choice were capable of improving positive mood on the day of influenza vaccination in older adults, compared to usual care. The rationale behind including a choice-based intervention arm followed evidence that choice may increase both perceived control and autonomy, which in turn was associated with better intervention outcomes (in terms of satisfaction, retention and mood outcomes) (Bartley et al., 2016; Beer et al., 2017; Carlisle et al., 2021). The secondary aim of the trial was to examine whether antibody responses to the influenza vaccine differed by trial arm (standardised intervention, choice intervention or usual care). The optimisation and validation of the immunoassays used to assess these antibody outcomes were described in *Chapter 4*.

The statistical analyses and findings from the For-ME Trial were outlined in Chapter 5 and are briefly restated below (see *Section 6.2*). In this final chapter, the implications of these findings will be discussed in relation to prior research. Research challenges and reflections encountered when conducting this research will be discussed, highlighting recommendations for alternative approaches if this work is to be replicated. Finally, the chapter will conclude with outlining future research propositions that can build on the research presented in this thesis.

## 6.2 Summary of For-ME Findings

The For-ME trial was a parallel, 3-armed randomised control trial conducted in 13 general practice surgeries within the East Midlands. A summary of the primary and secondary aims and their findings are outlined below, along with the results of any exploratory analyses.

(1) *Primary Aim:* To explore whether a standardised and/or choice intervention was capable of improving the positive mood of older adults prior to influenza vaccination compared with usual care, and to identify which intervention type was superior.

Both the standardised intervention and choice intervention statistically improved the positive mood of older adults, as well as usual care. Statistically significant changes in mood were indicated by the Affective Slider happiness subscale (the primary outcome measure), as well as the Affective Slider alertness subscale, DVAMs scales and SPANE scales. Group

allocation to an intervention group was found to be a significant predictor of post-intervention state affect compared with usual care. However, both interventions were found to be equally effective at improving positive mood.

(2) *Secondary Aim:* To explore whether group allocation predicted 4-weeks post-vaccination antibody responses.

Antibody responses to vaccination were measured via Enzyme-Linked Immunosorbent Assay (ELISA) and Hemagglutinin Inhibition Assay (HAI). Statistically significant changes in antibody levels following vaccination were observed for all influenza strains, measured by both ELISA and HAI. These results indicated that group allocation, to an intervention or usual care group, did not significantly predict 4-week post-vaccination antibody responses in terms of ELISA OD levels or in terms of the proportion of participants classified as seroprotected for either HAI threshold (≥40 and ≥160).

In addition to these primary and secondary aims, a series of exploratory analyses were carried out and are described below.

(1) To assess whether perceived stress and trait mood measured at baseline were associated with positive mood on the day of vaccination by investigating both pre- and post-intervention mood.

Analyses indicated that participants with high levels of perceived stress and negative trait affect, measured at baseline, scored lower pre-intervention positive state affect (for all measures) and higher negative state affect (SPANE negative). Additionally, participants with high positive trait affect at baseline scored higher pre-intervention positive state affect scores for all measures. However, these findings suggested that individuals' levels of perceived stress and negative trait affect measured at baseline did not influence how they responded to an intervention in terms of state affect. Higher scores of positive trait affect were found to increase post-intervention mood scores for the usual care and choice intervention groups, but not the standardised intervention group. These findings suggested that although baseline psychological factors are capable of predicting pre-intervention state affect, they do not play a role in predicting post-intervention state affect and therefore does not influence the effects of the interventions.

(2) To assess whether perceived stress and trait affect measured at baseline were associated with antibody responses to influenza vaccination.

High levels of perceived stress, negative trait affect and positive trait affect measured at baseline were not found to be predictors of antibody responses to influenza vaccination.

(3) To compare two different methods of measuring antibody responses to influenza vaccination.

Analyses indicated that ELISA and HAI were significantly correlated methods of measuring antibody responses to vaccination. However, Blind-Altman analyses established that these two different methods of measuring antibody responses to vaccination were statistically significantly different and therefore could not be utilised interchangeably.

## 6.3 Research Implications

### *6.3.1 Positive Mood Enhancing Interventions*

The primary aim of the For-ME trial was to examine whether two brief positive mood interventions (standardised and choice) improved the mood of older adults prior to vaccination when compared with usual care. Additionally, if these interventions were found to enhance positive mood, examine which intervention type was superior. To date there has been a modest amount of research into positive mood enhancing interventions that vary considerably in terms of target population and intervention type (see *Chapter 3*) (Davidson et al., 2003; Kiecolt-Glaser et al., 2008; Maryam Fakhrhosseini & Jeon, 2017; Vedhara et al., 2003, 2019). Prior to the research presented in this thesis, a pilot study conducted by Ayling et al. (2019) was the first brief positive mood enhancing standardised intervention that has been shown to improve the mood of older adults (*n*= 103) in a clinical setting prior to influenza vaccination. The research presented in this thesis is an alignment with these findings, whereby the change in state affect, measured by the primary outcome (Affective Slider happiness subscale) and the other five state affect measures (Affective Slider alertness subscale, DVAMS and SPANE), were found to be statistically significant for both intervention groups. Therefore, both interventions successfully enhanced the positive mood, and decreased the negative mood, of older adults immediately prior to influenza vaccination. These findings show that a 15-minute intervention, delivered in a clinical setting as part of standard vaccination care, is effective at improving mood. In comparison to the study by Ayling et al. (2019), the observed effect sizes were very similar (small to medium with partial ETA range of 0.017-0.063 in the For-ME trial and *d*= 0.6 in the Ayling pilot trial).

Whilst this is promising evidence to support the implementation of psychological interventions within healthcare settings, a limitation of these findings is that it remains unclear how long the effects of the intervention last could last for. Within the context of the

trial, researchers ensured participants received their influenza vaccination immediately after intervention engagement. This does not mean this will also be the case in the context of standard vaccination care if there are clinic delays and long patient queues, which is often the case. The known duration of positive mood induction means that it is possible the effects of the intervention could wear off in over-run and delayed clinics.

Another important consideration of these findings is that pre-intervention positive mood levels were found to be high across all three arms for all measures. There is a chance that the potential of these interventions to improve mood were limited by the ceiling effects of all state affect measures being high prior to intervention engagement. It may be that these interventions would be more effective for participants with low mood or in populations where low mood is particularly prevalent (discussed further in *Section 6.4.1*).

Some statistically significant changes were also observed in the usual care arm for both Affective Slider scales, both SPANE scales and the alertness DVAMS scale. Whilst we would not predict a significant change in mood score for the usual care group, these findings are not surprising due to the nature of the study and the target age of the population of interest. For this older adult population, getting involved in research was very enjoyable and exciting for them, whereby participants frequently expressed these feelings with researchers. This is perhaps unsurprising given the research that has identified high levels of loneliness for older adult populations, with 50% of individuals aged over 60 reported to be at a high risk of social isolation and over 70% will experience some sort of loneliness in later life (Fakoya et al., 2020). A possible explanation could be that research activities may have contributed to improved mood within the control arm and some participants in this arm even expressed enjoyment in the process informally to researchers. It is very likely that engagement with researchers and fellow participants could have been one of very few social interactions for them that week/month. Some research has been identified to investigate the engagement in research resulting in non-specific improvements in mood and has identified that participating in psychological research may temporarily amplify the experience of positive and negative emotions for participants (Dunsire, 1999). For example, Daugherty and Lawrence (1996) assessed the short-term effect of research participation on positive and negative feelings for a cohort of college students. Positive emotions were found to be endorsed significantly more than negative feelings, whereby personality traits could play a role in predisposing participants to positive or negative emotional reactions.

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Analyses indicated that group allocation to an intervention group, standardised or choice, was a significant predictor of post- intervention state affect compared with usual care. However, in contrast to the outlined hypothesis, the choice intervention was not shown to be significantly superior at predicting post-intervention state positive affect scores compared with the no choice, standardised (fixed-content) intervention. Whilst previous research has indicated that choice interventions enhance participant retention and adherence compared with no choice interventions (Lindhiem et al., 2014), there are mixed results about their improved ability to elicit mood changes (Carlisle et al., 2021). In a recent review by Carlisle et al. (2021), a total of 11 studies were examined to compare the impact of choice and non-choice interventions on mood. Whilst outcomes were mixed, with 7 of these studies favouring choice and 4 favouring no choice, they were mainly focused on negative affect measures, such as anxiety (Bartley et al., 2016), depression (Beer et al., 2017), distress (Yancy et al., 2015), worry (Brenes et al., 2020), nervousness (Chiviacowsky et al., 2012) and discomfort (Rose et al., 2014) with only 1 study investigating pleasure (Veitch & Newsham, 2000). Whilst some evidence in the review by Carlisle et al. indicated that choice interventions may result in greater improvements in mood, with point estimates favouring choice over no choice, confidence intervals were wide and were not statistically significant. It is unclear whether this means the provision of choice has no meaningful influence on mood change, or whether this is a result of sparse and divergent data making significant findings unlikely. The For-ME trial presents further evidence that the provision of choice may not be superior to no choice to improve mood outcomes for positive mood interventions. Limited evidence outlining the influence of choice interventions on mood highlights the need for more research in this area.

### *6.3.2 Trait Mood and Stress Influencing Intervention Effectiveness*

To take a closer look at participant trait mood, baseline perceived stress and PANAS positive and negative trait affect were measured to assess whether these factors influenced intervention effectiveness. The findings of exploratory analyses indicated baseline trait affect and perceived stress scores had little impact on the effectiveness of the interventions in terms of state affect responses. These findings suggested that although baseline psychological factors are capable of predicting pre-intervention state affect, they did not play a role in predicting post-intervention state affect and therefore did not influence the effects of the interventions. However, these participants did not experience noticeably high levels of perceived stress of negative trait affect, whereby scores for these baseline measures did not deviate from normative values that would be expected for this older adult population

(e.g., normative reported perceived stress scores were 12.0 compared with 12.5 in the For-ME trial, see *Section 5.3.3*). These findings suggest that normative perceived stress and trait mood levels were not important predictors of post-intervention state affect and subsequently did not mitigate the effects of intervention efficacy.

### *6.3.3 Psychological Factors and Influenza Vaccination Responses*

The secondary aim of the For-ME trial was to assess whether antibody responses to the influenza vaccination differed between those who received a positive mood intervention and usual care. In contrast to the proposed hypothesis, For-ME trial results indicated that group allocation did not significantly predict 4-week post-vaccination antibody responses to influenza vaccination in terms of ELISA OD levels or in terms of the proportion of participants classified as seroprotected for either threshold using the HAI assay (≥40 and ≥160).

Whilst there is prior research to suggest that positive mood can upregulate immunity (see *Chapter 2*), there is limited research on positive mood having immune enhancing effects on antibody responses to vaccination, particularly in older adult populations. The majority of research that has indicated the role of positive mood of immune modulation have primarily investigated trait affect, secretory IgA and cytokine responses, which is in contrast to the For-ME trial that investigated state affect and measured antibody responses to influenza vaccination (Barak, 2006; Marsland et al., 2007; Pressman & Cohen, 2005; Steptoe et al., 2009). This makes it challenging to compare this research with the For-ME findings but could be a potential explanation for the observed differences. For example, previous research by Marsland et al. (2006) found that trait positive affect of graduate students (ages 21-33 years) was associated with heightened antibody responses to hepatitis B vaccination. Whilst this research by Marsland et al. (2006) was important for highlighting the direct immune enhancing effects of positive mood, it focused on trait affect as opposed to state affect, looked at a much younger population, as well as a different vaccination type to those examined in the For-ME trial. It is very difficult to compare vaccination responses of younger and older adult populations due to older adults experiencing immune ageing (see *Chapter 1*). Older adults mounting considerably lower vaccination responses compared with young adults makes it an unfair comparison between these two populations, suggesting that these findings by Marsland et al. (2006) are not applicable to older adults. Very little research has focused on the positive affect of older adults in the context of immune responses, but there is some evidence that has provided some informative insight into these relationships (Costanzo et al., 2004). Similar to Marsland et al. (2006), this study by Costanzo et al. (2004) investigated trait affect, such as optimism and vigour, and found that high levels of these

measures were associated with greater cytokine responses. Specifically, T helper 1 cytokine responses (IFN-γ and IL-2) to vaccination were more robust in individuals with greater optimism, where the improvement in T helper 1 cytokine responses is particularly important due to the role of T helper 1 type responses in clearing viruses and preventing infection (Beňová et al., 2020).

Although the results presented in this thesis found that a 15-minute intervention is not sufficient to have a statistically significant impact on antibody responses 4-weeks post-vaccination with an adjuvanted vaccine, it is important to note that this trial was powered to assess between arm differences of mood outcomes primarily and not antibody outcomes. It is therefore unclear whether these results suggested that group allocation was not capable of predicting 4-weeks post-vaccination antibody responses or whether the role of mood was more important in the context of trait affect rather than state affect. Analyses were conducted to examine this (see *Section 6.3.4*).

### *6.3.4 Trait Affect, Stress and Vaccination Responses*

It may be that positive affect duration (trait instead of state) and magnitude are important factors to consider when investigating immune modulation. To investigate this, exploratory analyses were conducted to examine whether baseline measures, perceived stress and trait affect, were predictors of 4-weeks post-vaccination antibody responses. These analyses indicated that perceived stress and trait affect measures at baseline were also not significantly associated with 4-week post-vaccination antibody responses in terms of ELISA OD levels or in terms of the proportion of participants classified as seroprotected for either threshold. These findings are not consistent with the meta-analysis examining the relationship between stress and antibody responses to influenza vaccination, which found significant negative relationships between stress and antibody responses in studies conducted in older adults (Pedersen et al., 2009). However, much of the research contributing to this review focused on populations experiencing very high levels of chronic stress, such as elderly caregivers. For example, Kiecolt-Glaser et al. (1996) demonstrated that the down-regulation of immune responses to influenza vaccination, via antibody and cytokine responses, was associated with chronic stress in elderly caregivers. More of this research is reported in *Chapter 2*, which showed that chronic stress in older adult populations could have implications for their vulnerability to infection due to immune suppression (Kiecolt-Glaser et al., 1996; Phillips et al., 2006; Segerstrom et al., 2008; Thorpe et al., 2006; Vedhara et al., 1999). These For-ME trial results do not challenge these findings but instead may suggest that stressful situations must be of a sufficiently large magnitude to

act meaningfully on antibody responses to vaccination. It is possible that this also applies to trait affect, whereby the findings of this For-ME trial also found that positive and negative trait affect, measured at baseline, did not predict 4-weeks post-vaccination antibody responses. This is also contrary to previous research that has provided evidence for trait negative affect, such as depression, hindering immune responses to vaccination (Herbert & Cohen, 1993) and trait positive affect being associated with greater antibody responses to vaccination (Marsland et al., 2006). Whilst positive trait affect, such as dispositional optimism, has been found to have immune enhancing effects (Marsland et al., 2006), researchers have found that individuals with high levels of positive trait affect endorse more exercise and better overall sleep quality compared with low positive trait affect counterparts (Cohen et al., 2003b). These are important factors to take into consideration due to both sleep quality (Prather et al., 2021; D. J. Taylor et al., 2017) and physical exercise (Kohut et al., 2002) being shown to be associated with enhanced antibody responses to influenza vaccination.

Studies investigating the immunological effects of trait positive affect do not always take these factors into consideration, which can make it difficult to independently examine the relationship between positive affect and immunity. In the study by Marsland et al. (2006), researchers did assess the influences of positive affect and physical activity on vaccination responses and found they were largely independent, but did not examine sleep. This highlights one of the biggest issues with examining the immune benefits of positive affect due to the challenge of determining whether relationships are independent, or whether they are a consequence of a number of factors closely tied to positive affect, such as physical exercise and sleep. In sum, it seems that the diversity of research examining different affect types (trait or state), vaccination types, the range of immune measures and population types makes it difficult to understand the clinical impact, if any, of positive affect on the immune system and its ability to act on vaccination responses. It would be important for a larger scale study looking to closely investigating positive affect in older adult populations to take confounding factors, such a sleep quality and physical exercise, into consideration and to investigate populations screened for low mood who may be more sensitive to the effects of a mood enhancing intervention.

It is also perhaps worth noting that the measurement of these psychological factors (perceived stress and trait affect) at baseline required participants to report on single time-point retrospective questionnaires. This means that these measures rely on the ability of these participants to accurately and unbiasedly recall their experience over a prolonged

period of time (often weeks or months) and is unable to capture the inherent variability of these psychological states (Bolger et al., 2003; Conner & Barrett, 2012). For these reasons, momentary measures of affect are considered to be more reliable because they do not face the issue of memory bias and have been found to correlate more strongly with immune function compared with retrospective accounts of affect (Conner & Barrett, 2012). This is one of the strengths of the acute affect, pre- and post-intervention, measures that assessed how participants were feeling "in the moment". On the contrary, perceived stress and trait affect measures relied on these retrospective measures which are perhaps less reliable than other studies who use diary measures (multiple measures of the same psychological factor over a period of time). The observational study by Ayling et al. (2018) provides some evidence for the diary method of assessing perceived stress and trait affect. Researchers found a relationship between trait positive affect, but not trait negative affect or perceived stress, and H1N1 responses using a diary-based method. The methods of measuring trait affect and perceived stress could be one of the reasons the no effects of these psychological factors were found.

### *6.3.5 Assessing Vaccination Responses*

Another important consideration is whether alternative immune measures, in addition to antibody responses, could have served as a better indicators of vaccination responses and could have been more sensitive to the effects of the intervention. Whilst there are an array of methods for quantifying immune responses to vaccination, such as innate (e.g., macrophages), humoral (e.g., B cells that produce antibodies), cellular (e.g., T cells) and cytokine responses, the most frequently used is the measurement of antibody concentrations (Zimmermann et al., 2021). However, it has also been argued that antibody responses are not the best correlate of protection for influenza in older adults and that T cell-mediated responses could be better (McElhaney et al., 2006). McElhaney et al. (2006) gave some valuable insight about the importance of taking into consideration T cell responses to vaccination, where this study evaluated Th1 and Th2 (two T helper cell subtypes) cytokine ratios of IFNγ:IL-10. Findings indicated that laboratory diagnosed influenza patients had a lower ratio due to the under production of IFNγ and overproduction of IL-10, therefore reflecting a shift towards a Th2 response, which has been identified as a potential marker for determining risk for influenza illness. The impact of T cell production and activation is vital for aiding antibody production, especially in the context of vaccination. An important function of T helper cells is to activate B cells to produce antibodies specific to a foreign antigen, in this case via vaccination, which means that B cell production of

antibodies is dependent on the activation of specific T cell populations (Ahmed & Gray, 1996). Important T cell responses also involve cytotoxic T cells, also referred to as killer T cells, due to the ability of this T cell population type being able to directly recognise, bind to and destroy viruses. Cytotoxic T cell populations are important is the context of influenza vaccination due to the rapid rate of virus mutation (antigenic drift and shift, see *Chapter 1*) (Carrat & Flahault, 2007). This is because drastic viral mutation can result in immune evasion, whereby antibodies are unable to bind to the virus to neutralise it due to the virus becoming unrecognisable. However, cytotoxic T cells can recognise parts of amino acids that made up a virus and may be able to recognise and bind to some viruses despite them undergoing mutations. One notable study assessed both humoral-mediated and cellular-mediated immune responses to influenza vaccination. Wong et al. (2013) found that elderly caregivers, who were reported to have higher stress levels than matched controls, had decreased lymphocyte (cell-mediated) and cytokine immune responses to influenza vaccination. Crucially however, they observed no differences in humoral-mediated (antibody) immune responses to vaccination between caregivers and controls. This is an important comparison of immune findings because it suggests that the research in this field should look beyond humoral-immunity to also consider cellular immunity when investigating immune functioning.

This above evidence has highlighted the importance of taking other immune factors, such as T cell responses, into consideration when assessing vaccination responses and protection from infection for all population types. The assessment of T cell populations could prove to be particularly valuable when conducting studies on older adult populations due to them being subject to the effects of immune ageing. The effects of immunosenescence have been shown to result in thymic involution (shrinking), which can have a considerable impact on the immune dysregulation of T cell populations (see *Chapter 1*). Decreased thymic function, and consequently senescence of naïve T cell production, results in a reduced T cell repertoire, also known as clonal diversity of naïve T cells, and reduced chance of older adults mounting an immune response to a novel foreign antigen (Dewan et al., 2012). A range of important T cell subsets are impacted, including CD3+ (on T helper and cytotoxic T cells), CD4+ (on T helper cells) and CD8+ T cells (on cytotoxic T cells). These T cell responses are a vital component of the immune system when responding to vaccination and therefore highlights the importance of detecting both T helper and cytotoxic T cell responses, in addition to antibody responses, to get a more comprehensive overview of an individual's ability to mount an immune response to vaccination.

Whilst the findings of the For-ME trial did not indicate group allocation to be a predictor of antibody responses to vaccination, it raises the question about whether T cell and/or cytokine responses may have been more sensitive to the effects of the intervention. Cytokines have also been identified as an important component of the immune system by playing a critical role in the activation of cellular immune responses (Wurster & Grusby, 2004). Of particular importance in the context of positive affect is pro-inflammatory cytokine IL-6 that has been shown to be a key contributor to health outcomes, and has been frequently referenced throughout the stress and affect literature (Gentile et al., 2003; S. W. Lee et al., 1999; Lutgendorf et al., 2001; Mittwoch-Jaffe et al., 1995) (see *Chapter 2*). Individuals who experience high levels of chronic stress, such as caregiving, have been reported to upregulate IL-6, whereby this cytokine has been identified as a biomarker for infection and illness (Hayden et al., 1998). Conversely, trait positive affect has the opposite effect on the immune system, in terms of IL-6 regulation, compared to chronic stress. For example, Friedman et al. (2007) tested the hypothesis that psychological well-being could predict lower levels of pro-inflammatory factors and found that higher scores of positive relationships and purpose in life indicated lower IL-6 levels. The role of IL-6 as a key contributor to health outcomes, where it is readily cited within stress and more recently affect literature, makes it an important cytokine to monitor for future studies investigating positive affect and vaccination responses. It would also be interesting to investigate whether positive mood induction could be a potential option for reducing the high IL-6 levels of chronically stressed individuals as a method of combating its immunosuppressive effects, further highlighting the importance of measuring pro-inflammatory immune responses, such as IL-6.

Another possible explanation for the null effects of the interventions on antibody responses could be related to the highly robust vaccination responses observed. The 2019/20 influenza vaccination (administered as part of the For-ME trial) approved for older adults contained the adjuvant MF59, which is an oil-in-water emulsion of squalene oil. Squalene, a naturally occurring substance found in humans, animals, and plants, is highly purified for the vaccine manufacturing process and has been shown to enhance responses to vaccination (Domnich et al., 2017). The addition of this MF59 adjuvant influenza vaccination was found to be more effective than its non-adjuvanted comparator, with For-ME data indicating very high levels of 4-weeks post-vaccination seroprotection levels of >80% (89% for A/Brisbane/02/2018 (H1N1), 88% for A/Kansas/14/2017 (H3N2) and 95% for B/Colorado/06/2017 (B)) compared with a strain average of 33% in a previous study investigating the same population type (older

adults, >65 years) (Ayling et al., 2018). These very high vaccination response rates were much greater than predicted, and were also much higher than those predicted for older adult populations (vaccination response range of 18-50%) (Govaert et al., 1994; P. A. Gross et al., 1995). A likely explanation for these high vaccination response rates is partly due to the addition of an adjuvant, but may also be due to the fact that the virus strains did not drastically mutate and may be repeated strains found in previous influenza vaccinations. For example, the reason B/Colorado/06/2017 (B) seroprotection rates pre-vaccination were very high (79%) is because this strain was present within the influenza vaccination administered within the 2018/2019 season (the year prior to the For-ME trial) (Public Health England, 2018). A prerequisite for taking part in the For-ME trial was that participants were required to have received the previous year's vaccination. This was implemented as a study requirement to account for prior vaccination exposure to ensure participants had at least one prior exposure to the same influenza viral strains. It is not uncommon for vaccination research to account for vaccination history in this way, especially in the case of influenza vaccination, where there is a strong likelihood of prior viral exposure either naturally or via previous vaccination. However, the limitation of this is that pre-vaccination antibody levels for some participants were very high, with a very high percentage of participants in the trial meeting the ≥40 HAI seroprotection threshold before being vaccinated (63% for A/Brisbane/02/2018 (H1N1), 29% for A/Kansas/14/2017 (H3N2) and 79% for B/Colorado/06/2017 (B). High pre-vaccination seroprotection rates can make it challenging to assess post-vaccination responses. This was one of the main reasons behind exploring the ≥160 HAI threshold in addition to the conventional ≥40 HAI threshold. As predicted, the higher threshold resulted in lower pre- and post-vaccination seroprotection rates. Based on the conventional ≥40 HAI threshold, there was an increase in the proportion of participants classified as seroprotected from pre- to post-vaccination of 25% for H1N1, 59% for H3N2 and 16% for B. These changes in seroprotection rates were similar for the ≥160 HAI threshold, with an increase with rates of 34%, 55% and 24% for H1N1, H3N2 and B, respectively. Unsurprisingly, the lowest change in seroprotection change from pre- to post-vaccination was observed for the B/Colorado/06/2017 (B) strain due to its presence within the previous year's vaccination, and therefore meant participants had very high pre-vaccination seroprotection rates Although changes were not drastic, the addition of this more stringent ≥160 HAI threshold did appear to slightly increase seroprotection rates from pre- to post-vaccination for all vaccine components. These results therefore provided some supporting evidence for the debunking of the well-established HAI titre of ≥40 that has been identified

as being less reliable for older adult populations (Sicca et al., 2020; Trombetta et al., 2018). This previously conventional seroprotection threshold is no longer accepted as a threshold of protection by the European Medicines Agency for the influenza vaccination responses of older adult populations (European Medicines Agency, 2016). Regardless of the clinical thresholds used, these For-ME findings showed very robust responses to vaccination that could have outweighed the effects of a psychological adjuvant, such as a positive mood enhancing intervention.

Limited evidence within the For-ME trial findings indicated that age nor sex meaningfully influenced influenza vaccination responses. This limited evidence found that with each yearly increase in age, participants were 11.3% and 9.5% less likely to be classified as seroprotected for influenza strain A/Brisbane/02/2018 (H1N1) for thresholds ≥40 and ≥160, respectively. However, age was not shown to be a predictor of seroprotection for influenza strains A/Kansas/14/2017 (H3N2) or B/Colorado/06/2017 (B), as well as ELISA OD levels for any vaccine components. Whilst there is evidence for age impacting immunity, this research has conventionally compared differences between younger (<65 years) and older (>65 years) adult populations, whereas this For-ME trial had a very narrow age range of 65-85 years (mean= 72.89), which could be an explanation for these findings. Mixed evidence around the influence of sex and vaccination responses meant that there was no directional hypothesis. However, recently research has come to light that has found that older adult females are more susceptible to the effects of immune ageing compared to men (McGill & Benayoun, 2022). T cell dysfunction, as a result of thymic involution, is a commonly reported side effect of ageing (see *Chapter 1*). A recent study sought to investigate whether sex plays a role in T cell ageing and found that N-glycan branching increased with age in T cells from females to a greater degree than in those from age-matched males, whereby the largest difference in N-glycan branching with age between female and male individuals was found in $CD4^+$ T cell populations (McGill & Benayoun, 2022). N-glycans play an important role in regulating ligand production, such as galectin, that has been shown to bind to T cell receptors to form a lattice. The strength of the formed galectin lattice is dependent on N-glycan branching levels, which in turn effects clustering, signalling and endocytosis of surface receptors in T cells (Morgan et al., 2004). As a result of ageing, T cell function can be negatively affected by increased N-linked glycan branching, leading to inhibition of pro-inflammatory signals and promotion of anti-inflammatory signals (Lau et al., 2007). This research further highlights the importance of going beyond only assessing antibody responses when investigating vaccination responses

of older adults to examine T cell populations and to take a closer look at potential gender differences.

### *6.3.6 ELISA versus HAI*

The main focus of this thesis has been to investigate whether a standardised and/or choice intervention were capable of enhancing the positive mood of older adults, compared with usual care, and to examine whether group allocation was a predictor of 4-weeks post-vaccination antibody responses. The importance of vaccination research within the field of psychoneuroimmunology, particularly in light of the recent COVID-19 pandemic, means that is it of critical importance to determine how to best measure vaccination responses. As mentioned earlier in this chapter, the most commonly cited method of assessing vaccination responses is the quantification of vaccine-specific antibody concentrations (Zimmermann et al., 2021). This is largely due to that fact that antibodies play a critical role in protecting an individual from infectious disease, with IgG isotype antibodies serving as the primary focus of vaccination research. Approximately 70-75% of the serum antibody pool are IgG antibodies (subclasses include IgG1 (most abundant), IgG2, IgG3 and IgG4), which represents the primary mechanism for antibody-mediated protection from infectious diseases in humans (Krammer, 2019). IgA and IgM are occasionally used as correlates of vaccine-induced protection, with IgA being responsible for inducing mucosal responses and IgM being with first antibody isotype produced in response to virus exposure (Beyer et al., 1986).

Within this thesis, two different methods were utilised to assess antibody responses to influenza vaccination: ELISA and HAI. Conventionally, antibody responses to vaccination are measured via the HAI assay to classify individuals as seroprotected. A HAI titre threshold of ≥40 (1:40) classifies individuals as seroprotected, or clinically protected, based on a 50% reduction in disease (Hobson et al., 1972). Despite the HAI assay serving as the gold standard method of measuring antibody responses to vaccination, some of the vaccination literature has also reported the use of ELISA to assess antibody levels (Glaser et al., 1998; Kiecolt-Glaser et al., 1996; Vedhara et al., 1999). Within this thesis, an interesting avenue for exploration was the comparison of these two different methods to examine correlation and agreement, to assess whether these methods could be considered interchangeable. Analyses indicated that ELISA and HAI are significantly correlated methods of measuring antibody responses to vaccination, which is in alignment with the outlined hypothesis and is also supported by previous literature (Shekarchi et al., 1981; Sicca et al., 2020; Trombetta et al., 2018). Blind-Altman analyses established that these two different methods of measuring antibody responses to vaccination were statistically significantly different. These significant

differences meant that these two different methods could not be utilised interchangeably. These findings are consistent with the research conducted by Sicca et al. (2020) who found that whilst HAI and ELISA methods were highly correlated, they did not demonstrate any agreement or equivalency when measuring influenza vaccine-specific antibodies.

Typically, when carrying out an ELISA assay, higher OD values are indicative of greater clinical protection, although there are currently no accepted ELISA antibody thresholds that classify individuals as protected. This has previously been considered one of the strengths of the HAI over the ELISA assay, with the ≥40 HAI titre serving as a clinical protection threshold. However, over recent years the value of such thresholds have been questions, with some researchers demonstrating that hemagglutination inhibition titres do not always accurately predict whether an individual is protected from disease particularly for older adult populations (due to antibody avidity explained in more detail below) (J. Feng et al., 2009; Kirkpatrick et al., 2018). As a result, the European Medicines Agency have removed this as a clinical protection reference (European Medicines Agency, 2016). The removal of this clinical protection threshold then raises the question about whether alternative methods, such as ELISA, that are much easier and cheaper to run would be a sufficient method for assessing vaccination responses. Another reason researchers may want to explore alternative methods is because the HAI assay has also been noted to lack reproducibility and consistency, as shown by several inter-laboratory studies, due to there being difficulty around the standardisation of reagents, such as red blood cells from different species that differ in their ability to agglutinate influenza viruses (Sicca et al., 2020). Another limitation of HAI is that the assay has reduced sensitivity due to its reliance on serial dilutions. This means there is no gradation between HAI serial dilutions, which makes it difficult to distinguish between samples. Gradation to distinguish between samples is one of the strengths when using ELISA. In terms of the data output of both assays, ELISA absorbance (reported as the optical density) is measured objectively by an optical scanner, whereas the results of the HAI assay are read visually by the researcher. Manual readings require a degree of interpretation, which allows for potential errors and biases to occur, especially if researchers are unblinded to the research questions. That being said, ELISA relies on colorimetric change, which has a comparatively small dynamic range compared to more modern fluorescence-based assays (e.g., flow cytometry). This means that serum samples often require considerable dilution for the majority to fall within relatively narrow ELISA dynamic ranges (i.e., the linear range).

Although these two methods both measure antibody responses to vaccination, and are therefore correlated, it is perhaps not unsurprising to discover that they did not equate to

each other given they were not technically measuring exactly the same thing. The HAI assay relies on the characteristic property of red blood cells to form a lattice-like suspension structure in the presence of the influenza virus, a process known as hemagglutination. Lattice formation occurs as a result of hemagglutinin glycoproteins on the surface of the influenza viruses binding to the surface of red blood cells. Hemagglutinin is an influenza virus glycoprotein that consists of a globular head and stem (also known as the stalk). The assay detected antibodies (IgG and some residual IgM) that are able to bind the influenza viral haemagglutinin head domain and, in turn, inhibit virus-red blood cells hemagglutination. In this way the HAI assay is measuring the functionality of the influenza-specific antibodies by measuring their ability to bind to the specific influenza viruses of interest. On the other hand, the ELISA assay detected IgG class-specific antibodies that bind to the specified antigens of interest (A/Brisbane/02/2018 (H1N1), A/Kansas/14/2017 (H3N2) and B/Maryland/15/2016 (also referred to as B/Colorado/06/2017- like strain due to their structural similarities)). This assay detects both the haemagglutinin head and stalk domains of the influenza virus, whereas the HAI assay is unable to detect this stalk domain (Jacobsen et al., 2017; Trombetta et al., 2018). This stalk domain is effectively the opposite end of the haemagglutinin structure and includes two portions, contrasting the one portion head domain. The main differences between these domains is that the head domain evolves extremely quickly, due to antigenic drift and shift, whereas the stalk domain evolves slowly and is, therefore, more stable over time (Kirkpatrick et al., 2018). It is this rapid rate of mutation that makes the head domain more immunogenic compared with the stalk domain, and therefore makes it the major antigenic component on the surface of the virus. A large percentage of the antibodies generated after infection by influenza viruses are directed against specific antigenic sites located in the globular head domain of the hemagglutinin. This means that if the virus strains used in the vaccine are not antigenically well matched to the circulating viral strains, the antibodies produced will be unable to recognise them and will result in reduced vaccine effectiveness. Although most antibodies elicited by the hemagglutinin are strain specific and are directed against the globular head domain, some research has also indicated that neutralising antibodies are also able to bind to epitopes on the influenza virus hemagglutinin stalk domain (Kirkpatrick et al., 2018). Functionally, these domains are different, with the head domain mediating virus attachment to a host cell (via sialic acid binding pockets) and the stalk domain inducing pH-triggered membrane fusion between the viral envelope and the endosomal membrane of the cell (Kirkpatrick et al., 2018). These domains allow the virus to enter the host cell and release genetic material so that replication, transcription, and

translation of the viral genome can occur (e.g., viral infection). Research has found that the levels of virus-specific antibodies measured by ELISA are always greater than those measured by HAI (J. Feng et al., 2009). It has been suggested that this difference is due to antibody avidity, which is defined as the strength of antibody binding to an antigen. It has been proposed that low avidity antibodies are capable of binding well enough to be measured by ELISA but not enough to inhibit hemagglutination (de Bruijn et al., 1999). However, low avidity antibodies binding are not necessarily capable of having neutralising properties and therefore may not offer protection and cannot inhibit viral functions (Gulati et al., 2005; Laver et al., 1990). This suggests that the HAI more accurately assesses functional antibodies, and therefore is a better indication of an individual's protection from infection. However, research has found that in some cases, the HAI assay was found to be less sensitive than the ELISA assay and did not detect all neutralising antibodies (Remarque et al., 1998). This evidence implies that ELISA assays are over-estimating and HAI are under-estimating the quantity of functional (i.e., neutralising) antibodies and therefore the predicted likelihood of infection protection. It is possible that investigating antibody avidity could be more important than quantity, whereby a study found that avidity increased without a significant increase in antibody concentration (Gulati et al., 2005). This could be why some vaccines with low HAI titres demonstrate adequate protection and is the likely explanation behind the original implementation of the HAI seroprotection threshold.

It is important to note that influenza viruses have two types of glycoproteins on their surface: hemagglutinin (highly abundant) and neuraminidase (less abundant). Although less abundant, the neuraminidase glycoprotein has also been identified as an important target, with neuraminidase-specific antibodies being demonstrated to attenuate infection and prevent severe disease (Eichelberger & Wan, 2014). Similar to hemagglutinin, neuraminidase has a globular head and stalk domain, whereby specific mutations of the neuraminidase stalk domains have been shown to be associated with transmission rates. For example, length shortening of neuraminidase stalks (via the deletion of amino acids) has frequently been observed to be associated with increased lethality and transmission and therefore plays an important role in contributing towards pathogenicity (Li et al., 2014; McAuley et al., 2019; Stech et al., 2015). Most inactivated vaccinations contain both glycoprotein types, however the predominant contribution of hemagglutinin-specific antibodies to vaccine efficacy has made this specific glycoprotein the focus of immune assays, such as HAI, but does not consider the important neuraminidase component of an immune response. More recently, ELISA assays have been developed and tested to detect neuraminidase potency, whereby

ELISA outputs have been shown to correlate with immunogenicity (i.e., ability to provoke an immune response) (Wan et al., 2017). This highlights the importance of assessing influenza-specific antibodies that are capable of both detecting hemagglutinin and neuraminidase to be able to more accurately assess an individual's protection against infection. This is one of the strengths of ELISA, compared with HAI.

The lack of ELISA threshold, and HAI threshold recently being disregarded, it is difficult to comment on whether one method is superior over another for the quantification of antibody concentrations to vaccination. Both methods allow for the ability to assess change in antibody levels from pre- to post-vaccination, but are unable to accurately predict the likelihood of protection against infection. It is possible that one method of quantification is isolation is not sufficient, and perhaps a combination of HAI to assess neutralising, functional antibodies alongside and ELISA assay to assess neuraminidase antibodies would be most informative for assessing protection from infection. More research is required to better assess the different methods in terms of their ability to suggest seroprotection.

## 6.4 Reflections, Challenges and Recommendations

This section aims to describe some of the reflections and challenges experienced when conducting the research outlined within this thesis. It also outlines recommendations if this work was to be replicated.

### *6.4.1 Recruitment*

#### *6.4.1.1 Participant Recruitment*

Power calculations to detect a medium sized difference between experimental arms and control, with 90% power in separate one-tailed t-tests, determined that a sample of 253 participants in each experimental arm and 108 participants in the control arm was required. Therefore, the recruitment target for this trial was set at 650 participants in total, after allowing for reasonable attrition levels. This was quite a large number of participants to recruit within the relatively small timeframe in which influenza vaccinations are administered (typically from October to January). This meant it was important to start recruitment as early as possible. Although recruitment started in the summer months prior to the commencement of the influenza vaccination period, recruitment continued through to December due to the high volume of participants being recruited. This proved to be problematic on some occasions due to some participants being eager to get vaccinated as early as possible. As a result, some participants were already vaccinated when directly contacted to take part or were unhappy with being given vaccination dates in November or

December so would opt out of taking part to get vaccinated sooner. It is therefore recommended that future studies recruiting a large number of participants should start as early as possible in the calendar year to maximise the chances of achieving the desired sample size.

#### *6.4.1.2 General Practice Recruitment*

Prior to participant study recruitment, GP surgeries in the East Midlands area needed to be contacted and invited to take part. This was one of the most challenging aspects of running the study, whereby practice managers were often difficult to get in contact with and were often resistant to the idea of research being conducted within their clinical practices alongside influenza vaccination schedules. This was largely due to the fact that during the winter months, when influenza vaccinations are administered, GP surgeries were extremely busy and were often short staffed. It was extremely important for researchers to be flexible and adaptable to changes in study room locations and study date changes to ensure staff within the clinics were as happy as they could be to ensure the fluid flow of study activities. Unfortunately, recruitment across GP surgeries was not uniform, with expressions of interest from some practices being much lower than others. This meant at times in the recruitment process, it appeared that the total participant recruitment target of 650 may not be reached. To address this, an additional practice was approached and recruited as a site relatively late in the study period. This late change was successful in ameliorating recruitment concerns, but also contributed towards issues of lack of sample representativeness. For example, this GP practice was not originally approached due to its atypical over-65 demographic make-up of primarily ex-university employees or current staff.

#### *6.4.1.3 Sample Representativeness*

Ethnic sample representativeness of the For-ME trial was not achieved with 96.3% of the cohort being white, 93.4% living independently and over 75% having a school or university level of education. The need to increase diversity in clinical trials is an important issue to address due to the commonly reported disparities by race and ethnicity among those recruited for research (Gill & Redwood, 2013). For example, whilst both the standardised and choice interventions were found to statistically improve the positive mood of older adults compared with usual care, it is possible that under-represented races and ethnicities would not have been as responsive to the classically British music and comedy intervention content delivered. It is therefore impossible to determine whether minority races and ethnicities would have responded to the interventions in the same way as white, British participants. Language barriers could also be problematic for minority groups that may not

speak fluent English. For pragmatic reasons, participants who had an insufficient command of the English language were excluded from the trial due to there being difficulties of consenting and completing self-report questionnaires. This could be one of the explanations for the lack of sample representativeness, but unfortunately this issue cannot be easily solved due to there being a number of trial tasks that require listening, reading and writing in the English language.

Representation issues beyond intervention content and language barriers include the types of patients who are likely to be receptive to taking part in research. Research has found that a higher proportion of patients without depression are willing and eager to take part in research compared with depressed patients (Brown et al., 2019). Isolated groups and minorities, such as African Americans and Hispanics, have been shown to experience higher levels of major depression that are associated with greater health burdens compared with Whites (Dunlop et al., 2003). Based on the plethora of research that has suggested individuals with high levels of depression are more susceptible to infection and reduced vaccine efficacy (Afsar et al., 2009; Maes et al., 1994; Reiche et al., 2005), it is of particular importance for research aiming to improve vaccine efficacy to find avenues to recruit and target these minority populations. This also raises an important question about whether there is a greater room for positive mood improvement for populations who are not from socially advantageous and privileged backgrounds who have been highlighted to report higher levels of depression, and therefore the potential impact of interventions may be more effective in these populations. This links back to the discussion point raised earlier in this chapter, whereby it was stipulated that the high levels of pre-intervention mood created a ceiling effect to limit the potential impact of the interventions. This contributes towards the argument that these brief psychological interventions could be more effective at improving the mood participants with low mood.

In sum, the ethnic population sample presented within this thesis were not representative of the whole population and excluded a lot of important, under-represented groups. The main challenge when recruiting participants for clinical trials is finding avenues for accessing and engaging patients from minority races and ethnicities. Further research is needed to understand and address barriers to participation, particularly among under-represented groups in order to improve the validity and generalisability of clinical trial findings.

### *6.4.2 Usual Care as a Control Arm*

The pilot study, conducted by Ayling et al. (2019), that first investigated whether a positive mood intervention could enhance the positive mood of older adults and utilised a neutral intervention (time-matched video with neutral emotional content) as a control. However, this neutral intervention was not found to be a suitable control due to some participants reporting that they enjoyed the intervention, whereby participants allocated to this intervention were observed to have an increase in positive mood meaning this neutral intervention was an insufficient control. More importantly, even if it is possible for a more suitable neutral intervention could be developed, a neutral intervention comparator does not help to provide insight into whether the utilisation of an intervention is superior to usual care in the National Health Service. For these reasons, a usual care condition was implemented as the control arm or the For-ME trial. For usual care to have been replicated precisely, participants would have needed to have been escorted to a GP surgery waiting room for 15-minutes (the same duration as both intervention arms).This was originally the plan when For-ME clinic visits first started but proved to be challenging logistically for various reasons. In some clinics, the proximity of waiting rooms to the study room was too large and sometimes involved changing floors. The hindered mobility of some participants and the difficulty of researchers simultaneously coordinating several tasks in different rooms that were far apart made it impossible to stick to timings. In addition to this, utilisation of the study room was implemented to allow for greater control over external variables, such as waiting room music, televisions and posters, that are likely to vary between different clinics. All usual care participants were grouped in sessions together to ensure they did not have any second-hand intervention interaction (i.e., over see or hear another participant's tablet device who is engaging with an intervention), which assisted with replicating usual care. However, this is likely to have contributed towards the study effects that indicated statistically significant increases in the positive mood of older adults allocated to usual care. Whilst these increases were not as great as those observed for participants who were allocated to an intervention, it is clear the effects of taking part in research is enough to improve mood. Despite a statistically significant increase in mood in the usual care group, grouping usual care participants in a study room, as opposed to a neutral intervention or escorting participants in a GP surgery waiting room, was the best decision and did not influence the ability to detect significant intervention and usual care between arm differences in mood.

### *6.4.3 Vaccination Type*

For the 2018/19 influenza vaccination season, two vaccination types were approved for administration to older adults: the adjuvanted trivalent injected vaccine (aTIV) (commercially known as Fluad) (grown in eggs) and the quadrivalent injected vaccine (QIVc) (cell-grown) (Powis, 2019). On a purely scientific basis, selecting a non-adjuvanted vaccine was most appealing when considering the trial secondary aim of measuring antibody responses to vaccination. Adjuvants are added to vaccinations because they contain substances that boost an antigen's immunogenicity and, therefore accelerate vaccine responses. Depending on the type of adjuvant used, for which there is a broad spectrum to choose from, the quality of an immune response to vaccination can vary significantly. Different adjuvant types can change cytokine and chemokine profiles leading to the recruitment of different immune cells, and therefore have a downstream influence on antibody responses. Therefore, adjuvanted vaccinations add another dimension to consider when looking at immunological responses to vaccination (Tregoning et al., 2018). This made the use of the QIVc more desirable. However, this vaccine was also shown to be fractionally more expensive for clinical practices to purchase (£9.94 compared to £9.79 for the aTIV) (Powis, 2019). As a result, it was discovered that most of the clinical practices recruited for the trial had opted for the cheaper option (i.e., aTIV). Including both types of vaccination would have added additional variability, which would have made addressing the trial secondary aim of assessing vaccination specific antibody responses extremely challenging, and therefore meant one of the two needed to be chosen. Thus, primarily due to pragmatic reasons, it was decided that this trial would focus on using the aTIV vaccine.

As described earlier in this chapter, it has been argued that one of the reason the tested psychological interventions were not found to act meaningfully on vaccination outcomes in the findings of the For-ME study could be due to the fact the influenza vaccine investigated was adjuvanted. This is because the adjuvanted vaccine displayed considerably higher post-vaccination seroprotection rates (80%) compared with a similar population who received an unadjuvanted vaccine (30%) (Ayling et al., 2018). Of course, one option to avoid using adjuvanted vaccinations would be to assess another vaccination type all together. Whilst routine vaccinations, such as influenza vaccinations, allow researchers to easily “piggy back” onto usual vaccination care it adds an element of stress and pressure due to the previously mentioned restricted influenza vaccination timeframe. Occasional vaccines, such as hepatitis B, eliminates the challenge of recruiting participants within a limited timeframe. However, depending on the vaccination type and the number of older adults who are administered a

particular vaccination, it could be very time consuming to recruit sufficient numbers of participants. There are also additional hurdles to overcome with regards to ethics when investigating vaccinations that do not frequently form part of the standard care (e.g., keyhole limpet hemocyanin).

Ultimately, the focus of this research was to improve influenza vaccination responses of older adult due to their compromised immune systems. Influenza vaccination is the most commonly received vaccination for this population, and therefore makes most sense to investigate. Unfortunately, due to the above-mentioned issues, it would have been too challenging to use GP surgeries who were administering the QIVc and recruit a sufficient number of participants in the restricted timeframe. Perhaps, for studies with greater finances and resources to recruit GP surgeries outside of the East Midlands, it could be possible to investigate the more expensive, non-adjuvanted vaccination type.

### *6.4.4 Measuring Vaccination Responses*

To assess responses to vaccination as part of the For-ME trial, participant blood samples were obtained pre-vaccination and 4-weeks post-vaccination for ELISA and HAI analyses to determine vaccination-specific antibody levels. Whilst this 4-week post-vaccination timeframe has been suggested to be when peak antibody levels are reached and is often the convention cited in the literature (P. A. Gross et al., 1997; Siegrist, 2013), other research has found that older adults may reach an antibody peak later than 4-weeks (Kiecolt-Glaser et al., 1996; Wong et al., 2013). For example, a study conducted by Kiecolt-Glaser et al. (1996) found that a higher proportion of older adults who were administered an influenza vaccine were classified as being responders at 6-weeks post-vaccination compared with 4-weeks. This research is important to take into consideration because it demonstrates the value of extending the antibody measurement timeframe to ensure participants are not incorrectly classified as non-responders. This is a general limitation of the majority of the literature that investigates the influence of psychological factors on vaccination responses.

Future studies should consider either extending the post-vaccination timepoint from 4-weeks to 6-weeks, or should consider implementing more than one post-vaccination blood sampling timepoint. However, practically speaking the implementation of several post-vaccination blood sampling timepoints could prove to be particularly problematic for this older adult age group. Older adults are extremely difficult to obtain blood samples from due to there being a high risk of their veins collapsing upon the insertion of a blood sampling needle into their arm. They also often find the process of bleeding to be extremely painful

due to the build-up of scar tissue at the needle insertion site, which is due to the endurance of several blood samples over their lifetime. Neither of these are a health risk for participants, but it means that sometimes bleeding participants is not possible and is an uncomfortable experience for them. In some instances, blood sampling can be done with a smaller needle (i.e., butterfly needle) in the arm or top of the hand, which is less painful and allows for easier bleeding. However, these needles are much more expensive than regular bleeding needles and also make it difficult to collect 8mL of blood (this volume was the target for the For-ME trial) due to the small needle size making it an extremely slow process to collect the large volume required.

From a study logistics standpoint, an increase in post-vaccination blood sampling timepoints could prove to be very challenging, whereby participant recruitment could be more difficult to achieve and there is also an increased likelihood of loss to follow up due to older adults being resistant to blood sampling. An increase in blood sampling timepoints would also require increased support from GP surgeries, whereby researcher phlebotomy training has previously not been advised due to the challenge of bleeding this age group. This could make the recruitment of GP surgeries to take part in the research even more challenging because it requires more work and support from them to provide a nurse or healthcare assistant to facilitate blood sampling. There is also a cost-benefit profile to consider when determining whether to implement an additional bleeding timepoint. Additional costs would include an increase in inconvenience payment for participants, which could add up very quickly depending on the number of participants recruited. GP surgery costs would also increase due to the cost of consumables (e.g., blood sampling needles, additional blood tubes), nurse/ healthcare assistant time and room hire.

An easy solution for the above-mentioned issues would to be utilise finger prick blood sampling kits (capillary blood samples), as opposed to the venous blood sampling conducted in the For-ME trial. The main advantage of finger prick kits is that they are quick and easy to use, are relatively pain-free and only require 10uL of blood to measure antibody levels. Whilst it is possible for participants to do finger prick sampling themselves, there is room for error due to participants not completing saturating the absorbent collection swab, which is crucial for accurately measuring antibody levels. Research has indicated that venous and capillary blood sample results can differ when measuring some markers, such as capillary samples having significantly higher glucose levels compared with venous samples (Topping et al., 2019), but screening for antibody levels have been shown to be comparable between the two methods (Novello et al., 1996). Research has also found that despite reduced

cutaneous microvasculature of older adults that have the potential to affect the antibody measures of dried blood spots obtained from finger prick kits, sensitivity and specificity analyses found it to be a suitable alternative compared with venous blood sampling (Meyers et al., 2021). Additionally, no specialist training is required to use finger prick sampling kits meaning that researchers would be able to collect capillary blood samples themselves, reducing the burden for both participants and GP surgeries. However, an important weakness of finger prick blood sampling kits is that they are not suitable for assessing other aspects of the immune system (i.e., cellular immunity). As mentioned earlier in this chapter, it would be important for future studies to consider the measurement of cellular immune parameters, in addition to antibody levels, such as T cell and cytokine responses to vaccination that are not detectable using these finger prick blood sampling kits.

In sum, to be able to better measure vaccination responses, it is suggested that multiple post-vaccination blood sample timepoints should be added (4-weeks and 6-weeks) that can be measured via finger prick sampling kits. Additionally, immune parameters beyond antibody levels should also be assessed, whereby the value and importance of the obtained data around T cell and cytokine responses (mentioned in more detail earlier in this chapter) have the potential to outweigh the time and cost repercussions of venous blood sampling.

## 6.5 Future Research

### *6.5.1 Immunological Mechanisms of Positive Mood*

This proposed future study was one that was originally planned to be completed within the timeframe of my PhD. However, as mentioned within the COVID-19 impact statement near the start of this thesis, COVID-19 considerably impacted the ability to conduct research for a variety of reasons. Of particular importance to this proposed study was the indefinite pausing of non-COVID-19 related clinical research and university laboratory closure, which meant this planned study was unable to be conducted. It is proposed that a future study should be carried out to gain some insight into the immediate immune response after the induction of positive mood in older adults. The aim of this study would be to identify which markers of the innate immune system change immediately after inducing positive mood. These key components of the innate immune system include macrophages, NK cells, neutrophils, mast cells. Each participant recruited to take part in the study will be required to complete two separate clinic visits 1-week apart. They will complete one arm on the first visit (immediately after randomisation) and the second arm, a week later. These two arms are the standardised intervention and usual care control (the same as described for the For-ME trial). Participants

will be individually randomised to either start with the intervention or usual care condition. In both conditions, a cannula will be inserted into the participant and the first (baseline) blood sample taken. The cannula will be flushed with 5ml of normal saline after every blood sample is taken, to ensure further samples do not contain old blood from the line, as well as ensuring the cannula is still functioning properly. Before subsequent blood samples are taken, the first 1-2mL will be discarded to ensure the sample is not diluted from the saline flush, and to ensure good flow. 5-minutes after the baseline blood sample is collected, participants will individually view a 15-minute video package on a tablet device (standardised intervention), or be asked to sit in the consultation room for 15-minutes (usual care). Further blood samples will then be taken after 5-minutes and 10-minutes after intervention engagement/ waiting, followed by a final blood sample collected after 15-minutes (upon intervention/ waiting completion). A total of 4 blood samples for each participant will be collected, whereby cannulas will be removed after the final blood sample is taken.

Positive mood interventions have been highlighted in the literature to increase NK cell and neutrophil activity and have been shown to alter cytokine levels (Bittman et al., 2001; Koyama et al., 2009; Matsunaga et al., 2008; Takahashi et al., 2001). Therefore, blood samples will be assessed for a variety of immunological markers which form part of the innate immune response, including macrophages, neutrophils, NK cells, mast cells, histamine and cytokines. It is important to note that although cytokines are not specific to innate immunity because they are also produced my adaptive immune cells, it would be interesting to gain some insight into whether mood induction has an influence on cytokine profiles. Important cytokines that have been frequently cited within the literature investigating mood and immunity include IL-6, IFNγ and TNFα. Specifically, blood samples with be assessed to measure IFNγ, IL-1, IL-6, IL-10 and IL-2. IFNγ is a cytokine critical for both innate and adaptive immune responses, IL-1 plays a central role in the regulation of the immune and inflammatory response, IL-6 is a key pro-inflammatory cytokine, IL-10 is an anti-inflammatory cytokine and immune regulator and IL-2 regulates the activities of leucocytes (Wilson et al., 2007; Wurster & Grusby, 2004). Cytokines can be produced via the activation of several pathways, which could be triggered by immunological parameters relevant to the endocrine responses (i.e., cortisol and catecholamines) (Blalock et al., 1985; Elenkov et al., 2000). Blood samples will also be analysed to measure these endocrine markers. The purpose of measuring a broad spectrum of immune components it to gather information about how positive mood induction could be acting on the immune system. All of these immune cells can be measured via Enzyme-Linked Immunosorbent Assay (ELISA)

This mechanism study has the potential to provide some initial data to help design future, larger studies trying to identify the immune mechanisms responsible for creating an enhanced response to vaccination, in older adults with a positive mood at the time of vaccination.

## 6.6 Concluding Remarks

Influenza vaccination responses of older adult populations are extremely low and as a result have had a significant burden on the National Health Service due to large annual rates of influenza morbidity and mortality in the UK. The focus of the research presented within this thesis has explored the potential of psychological interventions to improve vaccination responses for this vulnerable population. Unfortunately, the research presented within this thesis has not been able to provide supporting evidence of brief positive mood enhancing interventions improving influenza vaccination responses in older adults. This does not mean that positive mood interventions are unable to act meaningfully on antibody-specific vaccination responses, but instead suggests that a larger scale study investigating additional immune parameters is required. A study to investigate the types of immune cells that are altered as a result of positive mood induction could help inform the types of immune parameters that should be explored for a larger scale study.

It could be argued that the research presented within this thesis is more important than ever with the most recent COVID-19 pandemic being indicated to have had catastrophic effects on older adult populations. This highlights the importance of the research being conducted to improve vaccination responses for this older adult population. There is the potential to apply psychological interventions to a variety of infection and vaccination studies that require the need for improvement for older adult populations, including influenza and COVID-19.

# References


Adelinis, J. D. (2005). *The role of choice versus preference: An analysis of why choice interventions work* [University of South Florida]. https://scholarcommons.usf.edu/etd/2769/

Ader, R., & Cohen, N. (1975). Behaviorally Conditioned Immunosuppression. *Psychosomatic Medicine*, *37*(4), 333–340. https://doi.org/10.1097/00006842-197507000-00007

Ader, R., Cohen, N., & Felten, D. (1995). Psychoneuroimmunology: interactions between the nervous system and the immune system. *The Lancet*, *345*(8942), 99–103. https://doi.org/10.1016/S0140-6736(95)90066-7

Afsar, B., Elsurer, R., Eyileten, T., Yilmaz, M. I., & Caglar, K. (2009). Antibody response following hepatitis B vaccination in dialysis patients: Does depression and life quality matter? *Vaccine*, *27*(42), 5865–5869. https://doi.org/10.1016/j.vaccine.2009.07.055

Ahmed, R., & Gray, D. (1996). Immunological memory and protective immunity: Understanding their relation. *Science*, *272*(5258), 54–60. https://doi.org/10.1126/science.272.5258.54

Aittomäki, S., & Pesu, M. (2014). Therapeutic targeting of the Jak/STAT pathway. *Basic & Clinical Pharmacology & Toxicology*, *114*(1), 18–23. https://doi.org/10.1111/BCPT.12164

Arck, P. C., Slominski, A., Theoharides, T. C., Peters, E. M. J., & Paus, R. (2006). Neuroimmunology of stress: Skin takes center stage. *Journal of Investigative Dermatology*, *126*(8), 1697–1704. https://doi.org/10.1038/sj.jid.5700104

Ayling, K., Fairclough, L., Buchanan, H., Wetherell, M. A., & Vedhara, K. (2019). Mood and influenza vaccination in older adults: A randomized controlled trial. *Health Psychology*, *38*(11), 984–996. https://doi.org/10.1037/hea0000786

Ayling, K., Fairclough, L., Tighe, P., Todd, I., Halliday, V., Garibaldi, J., Royal, S., Hamed, A., Buchanan, H., & Vedhara, K. (2018). Positive mood on the day of influenza vaccination predicts vaccine effectiveness: A prospective observational cohort study. *Brain, Behavior, and Immunity*, *67*, 314–323. https://doi.org/10.1016/j.bbi.2017.09.008

Ayling, K., Sunger, K., & Vedhara, K. (2020). Effects of brief mood-improving interventions on immunity: A systematic review and meta-analysis. *Psychosomatic Medicine*, *82*(1), 10–28. https://doi.org/10.1097/PSY.0000000000000760

Bachtiger, P., Adamson, A., Chow, J. J., Sisodia, R., Quint, J. K., & Peters, N. S. (2021). The impact of the COVID-19 pandemic on the uptake of influenza vaccine: UK-wide observational study. *JMIR Public Health and Surveillance*, *7*(4). https://doi.org/10.2196/26734

Bailey, E. S., Choi, J. Y., Fieldhouse, J. K., Borkenhagen, L. K., Zemke, J., Zhang, D., & Gray, G. C. (2018). The continual threat of influenza virus infections at the human–animal interface. *Evolution, Medicine, and Public Health*, *2018*(1), 192–198. https://doi.org/10.1093/emph/eoy013

Barak, Y. (2006). The immune system and happiness. *Autoimmunity Reviews*, *5*(8), 523–527. https://doi.org/10.1016/j.autrev.2006.02.010

Barrows, P., & Thomas, S. (2016). Development and validation of mood scales suitable for use in stroke patients with aphasia. In *University of Nottingham*.

Barrows, P., & Thomas, S. (2017). Assessment of mood in aphasia following stroke: validation of the Dynamic Visual Analogue Mood Scales (D-VAMS). *Clinical Rehabilitation*, *32*(1), 94–102. https://doi.org/10.1177/0269215517714590

Bartlett, D., Kaufman, D., & Smeltekop, R. (1993). The Effects of Music Listening and Perceived Sensory Experiences on the Immune System as Measured by Interleukin-1 and Cortisol. *Journal of Music Therapy*, *30*(4), 194–209. https://doi.org/10.1093/jmt/30.4.194

Bartley, H., Faasse, K., Horne, R., & Petrie, K. J. (2016). You Can't Always Get What You Want: The Influence of Choice on Nocebo and Placebo Responding. *Annals of Behavioral Medicine*, *50*(3), 445–451. https://doi.org/10.1007/s12160-016-9772-1

Baxter, D. (2007). Active and passive immunity, vaccine types, excipients and licensing. *Occupational Medicine*, *57*(8), 552–556. https://doi.org/10.1093/occmed/kqm110

Becoña, E., Vázquez, F. L., Fuentes, M. J., & del Carmen Lorenzo, M. (1998). Anxiety, affect, depression and cigarette consumption. *Personality and Individual Differences*, *26*(1), 113–119. https://doi.org/10.1016/S0191-8869(98)00129-9

Beer, N. J., Dimmock, J. A., Jackson, B., & Guelfi, K. J. (2017). Providing choice in exercise influences food intake at the subsequent meal. *Medicine and Science in Sports and Exercise*, *49*(10), 2110–2118. https://doi.org/10.1249/MSS.0000000000001330

Bennett, M. P., Zeller, J. M., Rosenberg, L., & McCann, J. (2003). The effect of mirthful laughter on stress and natural killer cell activity. *Alternative Therapies in Health and Medicine*, *9*(2), 38–42.

Beňová, K., Hancková, M., Koči, K., Kúdelová, M., & Betáková, T. (2020). T cells and their function in the immune response to viruses. *Acta Virologica*, *64*(2), 131–143. https://doi.org/10.4149/av_2020_203

Bernstein, E., Kaye, D., Abrutyn, E., Gross, P., Dorfman, M., & Murasko, D. M. (1999). Immune response to influenza vaccination in a large healthy elderly population. *Vaccine*, *17*(1), 82–94. https://doi.org/10.1016/S0264-410X(98)00117-0

Bertalanfy, L. Von. (1950). An Outline of Gneral System Theory. *The British Journal for the Philosophy of Science*, *1*(2), 134–165. https://doi.org/10.1093/bjps/I.2.134

Betella, A., & Verschure, P. F. M. J. (2016). The Affective Slider: A Digital Self-Assessment Scale for the Measurement of Human Emotions. *PLOS ONE*, *11*(2), e0148037. https://doi.org/10.1371/journal.pone.0148037

Beyer, W., Palache, A. M., Lüchters, G., Nauta, J., & Osterhaus, A. D. M. E. (2004). Seroprotection rate, mean fold increase, seroconversion rate: Which parameter adequately expresses seroresponse to influenza vaccination? *Virus Research*, *103*(1–2), 125–132. https://doi.org/10.1016/j.virusres.2004.02.024

Beyer, W., Van Der Logt, J. T. M., Van Beek, R., & Masurel, N. (1986). Immunoglobulin G, A and M response to influenza vaccination in different age groups: Effects of priming and boosting. *Journal of Hygiene*, *96*(3), 513–522. https://doi.org/10.1017/S0022172400066316

Bhutta, Z. A., Sommerfeld, J., Lassi, Z. S., Salam, R. A., & Das, J. K. (2014). Global burden, distribution, and interventions for infectious diseases of poverty. *Infectious Diseases of Poverty*, *3*(1), 21. https://doi.org/10.1186/2049-9957-3-21

Bigley, A. B., Rezvani, K., Chew, C., Sekine, T., Pistillo, M., Crucian, B., Bollard, C. M., & Simpson, R. J. (2014). Acute exercise preferentially redeploys NK-cells with a highly-differentiated phenotype and augments cytotoxicity against lymphoma and multiple myeloma target cells. *Brain, Behavior, and Immunity*, *39*, 160–171. https://doi.org/10.1016/j.bbi.2013.10.030

Bittman, B. B., Berk, L. S., Felten, D. L., Westengard, J., Simonton, O. C., Pappas, J., & Ninehouser, M. (2001). Composite effects of group drumming music therapy on modulation of neuroendocrine-immune parameters in normal subjects. *Alternative Therapies in Health and Medicine*, *7*(1), 38–47. https://www.researchgate.net/publication/12140102_Composite_Effects_of_Group_Drumming_Music_Therapy_on_Modulation_of_Neuroendocrine-immune_Parameters_in_Normal_Subjects

Blalock, J. E., Bost, K. L., & Smith, E. M. (1985). Neuroendocrine peptide hormones and their receptors in the immune system. production, processing and action. *Journal of Neuroimmunology*, *10*(1), 31–40. https://doi.org/10.1016/0165-5728(85)90032-3

Bland, J. M., & Altman, D. G. (1999). Measuring agreement in method comparison studies. *Statistical Methods in Medical Research*, *8*(2), 135–160. https://doi.org/10.1191/096228099673819272

Bolger, N., Davis, A., & Rafaeli, E. (2003). Diary Methods: Capturing Life as it is Lived. *Annual Review of Psychology*, *54*(1), 579–616. https://doi.org/10.1146/annurev.psych.54.101601.145030

Boraschi, D., & Italiani, P. (2014). Immunosenescence and vaccine failure in the elderly: Strategies for improving response. *Immunology Letters*, *162*(1), 346–353. https://doi.org/10.1016/j.imlet.2014.06.006

Borell-Carrió, F., Suchman, A. L., & Epstein, R. M. (2004). The biopsychosocial model 25 years later: Principles, practice, and scientific inquiry. *Annals of Family Medicine*, *2*(6), 576–582. https://doi.org/10.1370/afm.245

Bouvier, N. M., & Palese, P. (2008). The biology of influenza viruses. *Vaccine*, *26*, D49–D53. https://doi.org/10.1016/j.vaccine.2008.07.039

Boyce, W. T., Jensen, E. W., Cassel, J. C., Collier, A. M., Smith, A. H., & Ramey, C. T. (1977). Influence of life events and family routines on childhood respiratory tract illness. *Pediatrics*, *60*(4), 609–615. www.aappublications.org/news

Bradley, M. M., & Lang, P. J. (2017). International Affective Picture System. In *Encyclopedia of Personality and Individual Differences* (pp. 1–4). Springer International Publishing. https://doi.org/10.1007/978-3-319-28099-8_42-1

Breman, J. G., de Quadros, C. A., Dowdle, W. R., Foege, W. H., Henderson, D. A., John, T. J., & Levine, M. M. (2011). The Role of Research in Viral Disease Eradication and Elimination Programs: Lessons for Malaria Eradication. *PLoS Medicine*, *8*(1), e1000405. https://doi.org/10.1371/journal.pmed.1000405

Brenes, G. A., Divers, J., Miller, M. E., Anderson, A., Hargis, G., & Danhauer, S. C. (2020). Comparison of cognitive-behavioral therapy and yoga for the treatment of late-life worry: A randomized preference trial. *Depression and Anxiety*, *37*(12), 1194–1207. https://doi.org/10.1002/da.23107

Broadbent, D. E., Broadbent, M. H. P., Phillpotts, R. J., & Wallace, J. (1984). Some further

studies on the prediction of experimental colds in volunteers by psychological factors. *Journal of Psychosomatic Research*, *28*(6), 511–523. https://doi.org/10.1016/0022-3999(84)90085-0

Brown, J. S. L., Murphy, C., Kelly, J., & Goldsmith, K. (2019). How can we successfully recruit depressed people? Lessons learned in recruiting depressed participants to a multi-site trial of a brief depression intervention (the "CLASSIC" trial). *Trials*, *20*(1), 1–12. https://doi.org/10.1186/s13063-018-3033-5

Bucsek, M. J., Giridharan, T., MacDonald, C. R., Hylander, B. L., & Repasky, E. A. (2018). An overview of the role of sympathetic regulation of immune responses in infectious disease and autoimmunity. *International Journal of Hyperthermia*, *34*(2), 135–143. https://doi.org/10.1080/02656736.2017.1411621

Burns, V. E., Carroll, D., Ring, C., & Drayson, M. (2003). Antibody response to vaccination and psychosocial stress in humans: Relationships and mechanisms. *Vaccine*, *21*(19–20), 2523–2534. https://doi.org/10.1016/S0264-410X(03)00041-0

Burton, D. R. (2002). Antibodies, viruses and vaccines. *Nature Reviews Immunology*, *2*(9), 706–713. https://doi.org/10.1038/nri891

Butcher, S. K., Killampalli, V., Lascelles, D., Wang, K., Alpar, K. E., & Lord, J. M. (2005). Raised cortisol: DHEAS ratios in the elderly after injury: Potential impact upon neutrophil function and immunity. *Aging Cell*, *4*(6), 319–324. https://doi.org/10.1111/j.1474-9726.2005.00178.x

Callaway, B. E., & Callaway, E. (2020). Making sense of coronavirus mutations. *Nature*, *585*, 174–177. https://www.nature.com/articles/d41586-020-02544-6

Carlisle, S., Ayling, K., Jia, R., Buchanan, H., & Vedhara, K. (2021). The effect of choice interventions on retention-related, behavioural and mood outcomes: a systematic review with meta-analysis. *Health Psychology Review*, 1–37. https://doi.org/10.1080/17437199.2021.1962386

Carnethon, M. R., Kinder, L. S., Fair, J. M., Stafford, R. S., & Fortmann, S. P. (2003). Symptoms of depression as a risk factor for incident diabetes: Findings from the National Health and Nutrition Examination Epidemiologic Follow-up Study, 1971-1992. *American Journal of Epidemiology*, *158*(5), 416–423. https://doi.org/10.1093/aje/kwg172

Carpenter, L. L., Gawuga, C. E., Tyrka, A. R., Lee, J. K., Anderson, G. M., & Price, L. H. (2010). Association between plasma IL-6 response to acute stress and early-life adversity in healthy adults. *Neuropsychopharmacology*, *35*(13), 2617–2623. https://doi.org/10.1038/npp.2010.159

Carrat, F., & Flahault, A. (2007). Influenza vaccine: The challenge of antigenic drift. *Vaccine*, *25*(39–40), 6852–6862. https://doi.org/10.1016/j.vaccine.2007.07.027

Castés, M., Hagel, I., Palenque, M., Canelones, P., Corao, A., & Lynch, N. R. (1999). Immunological changes associated with clinical improvement of asthmatic children subjected to psychosocial intervention. *Brain, Behavior, and Immunity*, *13*(1), 1–13. https://doi.org/10.1006/brbi.1999.0551

Chi, R., Rock, M. T., & Neuzil, K. M. (2010). Immunogenicity and Safety of Intradermal Influenza Vaccination in Healthy Older Adults. *Clinical Infectious Diseases*, *50*(10), 1331–1338. https://doi.org/10.1086/652144

Chida, Y., & Steptoe, A. (2008). Positive psychological well-being and mortality: a quantitative review of prospective observational studies. *Psychosomatic Medicine*, *70*(7), 741–756. https://doi.org/10.1097/PSY.0B013E31818105BA

Chida, Y., & Vedhara, K. (2009). Adverse psychosocial factors predict poorer prognosis in HIV disease: A meta-analytic review of prospective investigations. *Brain, Behavior, and Immunity*, *23*(4), 434–445. https://doi.org/10.1016/j.bbi.2009.01.013

Chiviacowsky, S., Wulf, G., Lewthwaite, R., & Campos, T. (2012). Motor learning benefits of self-controlled practice in persons with Parkinson's disease. *Gait & Posture*, *35*(4), 601–605. https://doi.org/10.1016/j.gaitpost.2011.12.003

Chovatiya, R., & Silverberg, J. I. (2019). Pathophysiology of atopic dermatitis and psoriasis: Implications for management in children. *Children*, *6*(10). https://doi.org/10.3390/children6100108

Clem, A. (2011). Fundamentals of vaccine immunology. *Journal of Global Infectious Diseases*, *3*(1), 73. https://doi.org/10.4103/0974-777X.77299

Cohen, S., Alper, C. M., Doyle, W. J., Treanor, J. J., & Turner, R. B. (2006). Positive emotional style predicts resistance to illness after experimental exposure to rhinovirus or influenza A virus. *Psychosomatic Medicine*, *68*(6), 809–815. https://doi.org/10.1097/01.psy.0000245867.92364.3c

Cohen, S., Doyle, W. J., Skoner, D. P., Fireman, P., Gwaltney, J. M., & Newsom, J. T. (1995). State and Trait Negative Affect as Predictors of Objective and Subjective Symptoms of Respiratory Viral Infections. *Journal of Personality and Social Psychology*, *68*(1), 159–169. https://doi.org/10.1037/0022-3514.68.1.159

Cohen, S., Doyle, W. J., Skoner, D. P., Frank, E., Rabin, B. S., & Gwaltney, J. M. (1998). Types of stressors that increase susceptibility to the common cold in healthy adults. *Health Psychology*, *17*(3), 214–223. https://doi.org/10.1037/0278-6133.17.3.214

Cohen, S., Doyle, W. J., Turner, R., Alper, C. M., & Skoner, D. P. (2003a). Sociability and Susceptibility to the Common Cold. *Psychological Science*, *14*(5), 389–395. https://doi.org/10.1111/1467-9280.01452

Cohen, S., Doyle, W. J., Turner, R. B., Alper, C. M., & Skoner, D. P. (2003b). Emotional style and susceptibility to the common cold. *Psychosomatic Medicine*, *65*(4), 652–657. https://doi.org/10.1097/01.PSY.0000077508.57784.DA

Cohen, S., Janicki-Deverts, D., & Miller, G. (2007). Psychological Stress and Disease. *Journal of the American Medical Association*, *298*(14), 1685–1687.

Cohen, S., Kamarck, T., & Mermelstein, R. (1983). A Global Measure of Perceived Stress. *Journal of Health and Social Behavior*, *24*(4), 385. https://doi.org/10.2307/2136404

Cohen, S., Miller, G. E., & Rabin, B. S. (2001). Psychological Stress and Antibody Response to Immunization: A Critical Review of the Human Literature. *Psychosomatic Medicine*, *63*(1), 7–18. https://doi.org/10.1097/00006842-200101000-00002

Cohen, S., Tyrrell, D. A. J., & Smith, A. P. (1991). Psychological Stress and Susceptibility to the Common Cold. *New England Journal of Medicine*, *325*(9), 606–612. https://doi.org/10.1056/NEJM199108293250903

Cohen, S., Tyrrell, D. A. J., & Smith, A. P. (1993). Negative Life Events, Perceived Stress, Negative Affect, and Susceptibility to the Common Cold. *Journal of Personality and*

*Social Psychology*, *64*(1), 131–140. https://doi.org/10.1037/0022-3514.64.1.131

Cohen, S., & Williamson, G. (1988). Perceived stress in a probability sample of the United States. In *Newbury Park, CA: Sage* (pp. 31–67). https://psycnet.apa.org/record/1988-98838-002

Conner, T. S., & Barrett, L. F. (2012). Trends in Ambulatory Self-Report: The Role of Momentary Experience in Psychosomatic Medicine. *Psychosomatic Medicine*, *74*(4), 327. https://doi.org/10.1097/PSY.0B013E3182546F18

Consedine, N. S., & Moskowitz, J. T. (2007). The role of discrete emotions in health outcomes: A critical review. *Applied and Preventive Psychology*, *12*(2), 59–75. https://doi.org/10.1016/j.appsy.2007.09.001

Cordova, D. I., & Lepper, M. R. (1996). Intrinsic motivation and the process of learning: Beneficial effects of contextualization, personalization, and choice. *Journal of Educational Psychology*, *88*(4), 715–730. https://doi.org/10.1037/0022-0663.88.4.715

Costanzo, E. S., Lutgendorf, S. K., Kohut, M. L., Nisly, N., Rozeboom, K., Spooner, S., Benda, J. A., & McElhaney, J. E. (2004). Mood and cytokine response to influenza virus in older adults. *Journals of Gerontology - Series A Biological Sciences and Medical Sciences*, *59*(12), 1328–1333. https://doi.org/10.1093/gerona/59.12.1328

Coudeville, L., Andre, P., Bailleux, F., Weber, F., & Plotkin, S. (2010). A new approach to estimate vaccine efficacy based on immunogenicity data applied to influenza vaccines administered by the intradermal or intramuscular routes. *Human Vaccines*, *6*(10), 841–848. https://doi.org/10.4161/hv.6.10.12636

Dai, S., Mo, Y., Wang, Y., Xiang, B., Liao, Q., Zhou, M., Li, X., Li, Y., Xiong, W., Li, G., Guo, C., & Zeng, Z. (2020). Chronic Stress Promotes Cancer Development. *Frontiers in Oncology*, *10*(10), 1492. https://doi.org/10.3389/fonc.2020.01492

Daugherty, T. K., & Lawrence, J. W. (1996). Short-term effects of research participation on college men. *Journal of Psychology: Interdisciplinary and Applied*, *130*(1), 71–77. https://doi.org/10.1080/00223980.1996.9914989

Davidson, R. J., Kabat-Zinn, J., Schumacher, J., Rosenkranz, M., Muller, D., Santorelli, S. F., Urbanowski, F., Harrington, A., Bonus, K., & Sheridan, J. F. (2003). Alterations in brain and immune function produced by mindfulness meditation. *Psychosomatic Medicine*, *65*(4), 564–570. https://doi.org/10.1097/01.PSY.0000077505.67574.E3

de Bruijn, I. A., Remarque, E. J., Jol-van der Zijde, C. M., van Tol, M. J. D., Westendorp, R. G. J., & Knook, D. L. (1999). Quality and Quantity of the Humoral Immune Response in Healthy Elderly and Young Subjects after Annually Repeated Influenza Vaccination. *The Journal of Infectious Diseases*, *179*(1), 31–36. https://doi.org/10.1086/314540

de Jong, J. C., Palache, A. M., Beyer, W. E., Rimmelzwaan, G. F., Boon, A. C., & Osterhaus, A. D. (2003). Hemagglutination-inhibition assay for influenza virus subtype identification and the detection and quantitation of serum antibodies to influenza virus. *Developments in Biologicals*, *115*, 63–73.

Dedovic, K., D'Aguiar, C., & Pruessner, J. C. (2009). What stress does to your brain: A review of neuroimaging studies. *Canadian Journal of Psychiatry*, *54*(1), 6–15. https://doi.org/10.1177/070674370905400104

Dedovic, K., Renwick, R., Mahani, N. K., Engert, V., Lupien, S. J., & Pruessner, J. C. (2005). The Montreal Imaging Stress Task: Using functional imaging to investigate the effects

of perceiving and processing psychosocial stress in the human brain. *Journal of Psychiatry and Neuroscience*, *30*(5), 319–325.

Deniz, G., Erten, G., Kücüksezer, U. C., Kocacik, D., Karagiannidis, C., Aktas, E., Akdis, C. A., & Akdis, M. (2008). Regulatory NK Cells Suppress Antigen-Specific T Cell Responses. *The Journal of Immunology*, *180*(2), 850–857. https://doi.org/10.4049/jimmunol.180.2.850

Dewan, S. K., Zheng, S., Xia, S., & Bill, K. (2012). Senescent remodeling of the immune system and its contribution to the predisposition of the elderly to infections. *Chinese Medical Journal*, *125*(18), 3325–3331. https://doi.org/10.3760/cma.j.issn.0366-6999.2012.18.023

DeWitt, J. C., & Luebke, R. W. (2015). Immunological Aging. In *Reference Module in Biomedical Sciences*. Elsevier. https://doi.org/10.1016/B978-0-12-801238-3.02006-7

Dhabhar, F. (2008). Enhancing versus Suppressive Effects of Stress on Immune Function: Implications for Immunoprotection versus Immunopathology. *Allergy, Asthma & Clinical Immunology*, *4*(1), 1–10. https://doi.org/10.1186/1710-1492-4-1-2

Dhabhar, F. S. (2000). Acute stress enhances while chronic stress suppresses skin immunity: The role of stress hormones and leukocyte trafficking. *Annals of the New York Academy of Sciences*, *917*, 876–893. https://doi.org/10.1111/j.1749-6632.2000.tb05454.x

Dhabhar, F. S., Satoskar, A. R., Bluethmann, H., David, J. R., & McEwen, B. S. (2000). Stress-induced enhancement of skin immune function: A role for γ interferon. *Proceedings of the National Academy of Sciences of the United States of America*, *97*(6), 2846–2851. https://doi.org/10.1073/pnas.050569397

Dhabhar, F. S., & Viswanathan, K. (2005). Short-term stress experienced at time of immunization induces a long-lasting increase in immunologic memory. *American Journal of Physiology - Regulatory Integrative and Comparative Physiology*, *289*(3), 58. https://doi.org/10.1152/ajpregu.00145.2005

Diehl, S., & Rincón, M. (2002). The two faces of IL-6 on Th1/Th2 differentiation. *Molecular Immunology*, *39*(9), 531–536. https://doi.org/10.1016/S0161-5890(02)00210-9

Diener, E., Wirtz, D., Tov, W., Kim-Prieto, C., Choi, D. won, Oishi, S., & Biswas-Diener, R. (2009). New well-being measures: Short scales to assess flourishing and positive and negative feelings. *Social Indicators Research*, *39*, 247–266. https://doi.org/10.1007/s11205-009-9493-y

Dockray, S., & Steptoe, A. (2010). Positive affect and psychobiological processes. *Neuroscience and Biobehavioral Reviews*, *35*(1), 69–75. https://doi.org/10.1016/j.neubiorev.2010.01.006

Domnich, A., Arata, L., Amicizia, D., Puig-Barberà, J., Gasparini, R., & Panatto, D. (2017). Effectiveness of MF59-adjuvanted seasonal influenza vaccine in the elderly: A systematic review and meta-analysis. *Vaccine*, *35*(4), 513–520. https://doi.org/10.1016/j.vaccine.2016.12.011

Donker, F. J. S. (2000). Cardiac rehabilitation: A review of current developments. *Clinical Psychology Review*, *20*(7), 923–943. https://doi.org/10.1016/S0272-7358(99)00016-1

Donoyama, N., Munakata, T., & Shibasaki, M. (2010). Effects of Anma therapy (traditional Japanese massage) on body and mind. *Journal of Bodywork and Movement Therapies*,

*14*(1), 55–64. https://doi.org/10.1016/j.jbmt.2008.06.007

Donoyama, N., & Shibasaki, M. (2010). Differences in practitioners' proficiency affect the effectiveness of massage therapy on physical and psychological states. *Journal of Bodywork and Movement Therapies*, *14*(3), 239–244. https://doi.org/10.1016/j.jbmt.2009.01.007

Doyle, W. J., Gentile, D. A., & Cohen, S. (2006). Emotional style, nasal cytokines, and illness expression after experimental rhinovirus exposure. *Brain, Behavior, and Immunity*, *20*(2), 175–181. https://doi.org/10.1016/j.bbi.2005.05.005

Drela, N., Kozdron, E., & Szczypiorski, P. (2004). Moderate exercise may attenuate some aspects of immunosenescence. *BMC Geriatrics*, *4*(29), 8. https://doi.org/10.1186/1471-2318-4-8

DuBois, F. S., & Foley, J. O. (1936). Experimental studies on the vagus and spinal accessory nerves in the cat. *The Anatomical Record*, *64*(3), 285–307. https://doi.org/10.1002/ar.1090640303

Dunlop, D. D., Song, J., Lyons, J. S., Manheim, L. M., & Chang, R. W. (2003). Racial/Ethnic Differences in Rates of Depression among Preretirement Adults. *American Journal of Public Health*, *93*(11), 1945–1952. https://doi.org/10.2105/AJPH.93.11.1945

Dunsire, M. (1999). Emotion altering effects of research participation. In *Theses: Doctorates and Masters*. https://ro.ecu.edu.au/theses/1219

Edwards, K. M., Burns, V. E., Reynolds, T., Carroll, D., Drayson, M., & Ring, C. (2006). Acute stress exposure prior to influenza vaccination enhances antibody response in women. *Brain, Behavior, and Immunity*, *20*(2), 159–168. https://doi.org/10.1016/j.bbi.2005.07.001

Eichelberger, M. C., & Wan, H. (2014). Influenza Neuraminidase as a Vaccine Antigen. In *Current Topics in Microbiology and Immunology* (Vol. 386, pp. 275–299). Curr Top Microbiol Immunol. https://doi.org/10.1007/82_2014_398

Elenkov, I. J., Wilder, R. L., Chrousos, G. P., & Vizi, E. S. (2000). The sympathetic nerve - An integrative interface between two supersystems: The brain and the immune system. *Pharmacological Reviews*, *52*(4), 595–638.

Emery, C. F., Kiecolt-Glaser, J. K., Glaser, R., Malarkey, W. B., & Frid, D. J. (2005). Exercise accelerates wound healing among healthy older adults: A preliminary investigation. *Journals of Gerontology - Series A Biological Sciences and Medical Sciences*, *60*(11), 1432–1436. https://doi.org/10.1093/gerona/60.11.1432

Engel, G. (1960). A Unified Concept of Health and Disease. *IRE Transactions on Medical Electronics*, *3*(4), 459485. https://doi.org/10.1109/IRET-ME.1960.5008004

Engel, G. (1977). The need for a new medical model: a challenge for biomedicine. *Science*, *196*(4286), 129–136. https://doi.org/10.1126/science.847460

Ershler, W. B., & Keller, E. T. (2000). Age-Associated Increased Interleukin-6 Gene Expression, Late-Life Diseases, and Frailty. *Annual Review of Medicine*, *51*(1), 245–270. https://doi.org/10.1146/annurev.med.51.1.245

Eskandari, F., & Sternberg, E. M. (2002). Neural-immune interactions in health and disease. *Annals of the New York Academy of Sciences*, *966*(1), 20–27. https://doi.org/10.1111/j.1749-6632.2002.tb04198.x

European Medicines Agency. (2016). *Committee for Medicinal Products for Human Use Guideline on Influenza Vaccines Non-clinical and Clinical Module*. www.ema.europa.eu/contact

Evans, P., Bristow, M., Hucklebridge, F., Clow, A., & Walters, N. (1993). The relationship between secretory immunity, mood and life-events. *British Journal of Clinical Psychology*, *32*(2), 227–236. https://doi.org/10.1111/j.2044-8260.1993.tb01050.x

Fakoya, O. A., McCorry, N. K., & Donnelly, M. (2020). Loneliness and social isolation interventions for older adults: A scoping review of reviews. *BMC Public Health*, *20*(1), 1–14. https://doi.org/10.1186/s12889-020-8251-6

Farre, A., & Rapley, T. (2017). The New Old (and Old New) Medical Model: Four Decades Navigating the Biomedical and Psychosocial Understandings of Health and Illness. *Healthcare*, *5*(4), 88. https://doi.org/10.3390/healthcare5040088

Feng, J., Gulati, U., Zhang, X., Keitel, W. A., Thompson, D. M., James, J. A., Thompson, L. F., & Air, G. M. (2009). Antibody quantity versus quality after influenza vaccination. *Vaccine*, *27*(45), 6358–6362. https://doi.org/10.1016/j.vaccine.2009.06.090

Feng, L., Shay, D. K., Jiang, Y., Zhou, H., Chen, X., Zheng, Y., Jiang, L., Zhang, Q., Lin, H., Wang, S., Ying, Y., Xu, Y., Wang, N., Feng, Z., Viboud, C., Yang, W., & Yu, H. (2012). Influenza-associated mortality in temperate and subtropical Chinese cities, 2003–2008. *Bulletin of the World Health Organization*, *90*(4), 279-288B. https://doi.org/10.2471/BLT.11.096958

Folkman, S. (2013). Stress: Appraisal and Coping. In *Encyclopedia of Behavioral Medicine* (pp. 1913–1915). Springer New York. https://doi.org/10.1007/978-1-4419-1005-9_215

Fonkwo, P. N. (2008). Pricing infectious disease. *EMBO Reports*, *9*(S1). https://doi.org/10.1038/embor.2008.110

Fortes, C., Farchi, S., Forastiere, F., Agabiti, N., Pacifici, R., Zuccaro, P., Perucci, C. A., & Ebrahim, S. (2003). Depressive symptoms lead to impaired cellular immune response. *Psychotherapy and Psychosomatics*, *72*(5), 253–260. https://doi.org/10.1159/000071896

Franchimont, D. (2004). Overview of the actions of glucocorticoids on the immune response: A good model to characterize new pathways of immunosuppression for new treatment strategies. *Annals of the New York Academy of Sciences*, *1024*, 124–137. https://doi.org/10.1196/annals.1321.009

Friedman, E. M., Hayney, M., Love, G. D., Singer, B. H., & Ryff, C. D. (2007). Plasma interleukin-6 and soluble IL-6 receptors are associated with psychological well-being in aging women. *Health Psychology*, *26*(3), 305–313. https://doi.org/10.1037/0278-6133.26.3.305

Galic, M. A., Riazi, K., & Pittman, Q. J. (2012). Cytokines and brain excitability. *Frontiers in Neuroendocrinology*, *33*(1), 116–125. https://doi.org/10.1016/j.yfrne.2011.12.002

Ganta, C. K., Blecha, F., Ganta, R. R., Helwig, B. G., Parimi, S., Lu, N., Fels, R. J., Musch, T. I., & Kenney, M. J. (2004). Hyperthermia-enhanced splenic cytokine gene expression is mediated by the sympathetic nervous system. *Physiological Genomics*, *19*(2), 175–183. https://doi.org/10.1152/physiolgenomics.00109.2004

Gavazzi, G., Herrmann, F., & Krause, K. H. (2004). Aging and infectious diseases in the

developing world. *Clinical Infectious Diseases*, *39*(1), 83–91. https://doi.org/10.1086/421559

Gentile, D. A., Villalobos, E., Angelini, B., & Skoner, D. (2003). Cytokine levels during symptomatic viral upper respiratory tract infection. *Annals of Allergy, Asthma and Immunology*, *91*(4), 362–367. https://doi.org/10.1016/S1081-1206(10)61683-3

George, D., & Mallery, M. (2010). *SPSS for Windows Step by Step: A Simple Guide and Reference, 17.0 update* (10th ed.). Pearson : Allyn & Bacon.

Giefing-Kröll, C., Berger, P., Lepperdinger, G., & Grubeck-Loebenstein, B. (2015). How sex and age affect immune responses, susceptibility to infections, and response to vaccination. *Aging Cell*, *14*(3), 309. https://doi.org/10.1111/ACEL.12326

Gill, P. S., & Redwood, S. (2013). Editorials: Under-representation of minority ethnic groups in research-call for action. *British Journal of General Practice*, *63*(612), 342–343. https://doi.org/10.3399/bjgp13X668456

Ginaldi, L., Loreto, M. F., Corsi, M. P., Modesti, M., & De Martinis, M. (2001). Immunosenescence and infectious diseases. *Microbes and Infection*, *3*(10), 851–857. https://doi.org/10.1016/S1286-4579(01)01443-5

Glaser, R., & Kiecolt-Glaser, J. K. (2005). Stress-induced immune dysfunction: implications for health. *Nature Reviews Immunology*, *5*(3), 243–251. https://doi.org/10.1038/nri1571

Glaser, R., Kiecolt-Glaser, J. K., Bonneau, R. H., Malarkey, W., Kennedy, S., & Hughes, J. (1992). Stress-induced modulation of the immune response to recombinant hepatitis B vaccine. *Psychosomatic Medicine*, *54*(1), 22–29. https://doi.org/10.1097/00006842-199201000-00005

Glaser, R., Kiecolt-Glaser, J. K., Malarky, W. B., & Sheridan, J. F. (1998). The Influence of Psychological Stress on the Immune Response to Vaccines a. *Annals of the New York Academy of Sciences*, *840*(1), 649–655. https://doi.org/10.1111/j.1749-6632.1998.tb09603.x

Glaser, R., Rice, J., Sheridan, J., Fertel, R., Stout, J., Speicher, C., Pinsky, D., Kotur, M., Post, A., Beck, M., & Kiecolt-Glaser, J. (1987). Stress-related immune suppression: Health implications. *Brain, Behavior, and Immunity*, *1*(1), 7–20. https://doi.org/10.1016/0889-1591(87)90002-X

Glaser, R., Sheridan, J., Malarkey, W. B., MacCallum, R. C., & Kiecolt-Glaser, J. K. (2000). Chronic stress modulates the immune response to a pneumococcal pneumoaia vaccine. *Psychosomatic Medicine*, *62*(6), 804–807. https://doi.org/10.1097/00006842-200011000-00010

Globerson, A., & Effros, R. B. (2000). Ageing of lymphocytes and lymphocytes in the aged. *Immunology Today*, *21*(10), 515–521. https://doi.org/10.1016/S0167-5699(00)01714-X

Goehler, L. E., Relton, J. K., Dripps, D., Kiechle, R., Tartaglia, N., Maier, S. F., & Watkins, L. R. (1997). Vagal paraganglia bind biotinylated interleukin-1 receptor antagonist: A possible mechanism for immune-to-brain communication. *Brain Research Bulletin*, *43*(3), 357–364. https://doi.org/10.1016/S0361-9230(97)00020-8

Goodwin, K., Viboud, C., & Simonsen, L. (2006). Antibody response to influenza vaccination in the elderly: A quantitative review. *Vaccine*, *24*(8), 1159–1169.

https://doi.org/10.1016/j.vaccine.2005.08.105

Govaert, T. M. E. (1994). The Efficacy of Influenza Vaccination in Elderly Individuals. *JAMA*, *272*(21), 1661. https://doi.org/10.1001/jama.1994.03520210045030

Govaert, T. M. E., Dinant, G. J., Knottnerus, J. A., Masurel, N., Sprenger, M. J. W., & Thijs, C. T. M. C. N. (1994). The Efficacy of Influenza Vaccination in Elderly Individuals: A Randomized Double-blind Placebo-Controlled Trial. *JAMA: The Journal of the American Medical Association*, *272*(21), 1661–1665. https://doi.org/10.1001/jama.1994.03520210045030

Graham, N. M. H., Douglas, R. M., & Ryan, P. (1986). Stress and acute respiratory infection. *American Journal of Epidemiology*, *124*(3), 389–401. https://doi.org/10.1093/oxfordjournals.aje.a114409

Grant, R. W., Mariani, R. A., Vieira, V. J., Fleshner, M., Smith, T. P., Keylock, K. T., Lowder, T. W., McAuley, E., Hu, L., Chapman-Novakofski, K., & Woods, J. A. (2008). Cardiovascular exercise intervention improves the primary antibody response to keyhole limpet hemocyanin (KLH) in previously sedentary older adults. *Brain, Behavior, and Immunity*, *22*(6), 923–932. https://doi.org/10.1016/j.bbi.2008.01.006

Grebe, K. M., Hickman, H. D., Irvine, K. R., Takeda, K., Bennink, J. R., & Yewdell, J. W. (2009). Sympathetic nervous system control of anti-influenza CD8 + T cell responses. *Proceedings of the National Academy of Sciences of the United States of America*, *106*(13), 5300–5395. https://doi.org/10.1073/pnas.0808851106

Greenwood, B. (2014). The contribution of vaccination to global health: past, present and future. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *369*(1645), 20130433. https://doi.org/10.1098/rstb.2013.0433

Groer, M., Mozingo, J., Droppleman, P., Davis, M., Jolly, M. L., Boynton, M., Davis, K., & Kay, S. (1994). Measures of salivary secretory immunoglobulin A and state anxiety after a nursing back rub. *Applied Nursing Research*, *7*(1), 2–6. https://doi.org/10.1016/0897-1897(94)90013-2

Gross, J., & Levenson, R. (1995). Emotion Elicitation using Films. *Cognition and Emotion*, *9*(1), 87–108. https://doi.org/10.1080/02699939508408966

Gross, P. A., Hermogenes, A. W., Sacks, H. S., Lau, J., & Levandowski, R. A. (1995). The efficacy of influenza vaccine in elderly persons. A meta-analysis and review of the literature. *Annals of Internal Medicine*, *123*(7), 518–527. https://doi.org/10.7326/0003-4819-123-7-199510010-00008

Gross, P. A., Russo, C., Dran, S., Cataruozolo, P., Munk, G., & Lancey, S. C. (1997). Time to earliest peak serum antibody response to influenza vaccine in the elderly. *Clinical Diagnostic Laboratory Immunology*, *4*(4), 491–492. https://doi.org/10.1128/cdli.4.4.491-492.1997

Gulati, U., Kumari, K., Wu, W., Keitel, W. A., & Air, G. M. (2005). Amount and avidity of serum antibodies against native glycoproteins and denatured virus after repeated influenza whole-virus vaccination. *Vaccine*, *23*(11), 1414–1425. https://doi.org/10.1016/j.vaccine.2004.08.053

Haq, K., & McElhaney, J. E. (2014). Immunosenescence: influenza vaccination and the elderly. *Current Opinion in Immunology*, *29*, 38–42. https://doi.org/10.1016/j.coi.2014.03.008

Hayden, F. G., Fritz, R. S., Lobo, M. C., Alvord, W. G., Strober, W., & Straus, S. E. (1998). Local and systemic cytokine responses during experimental human influenza A virus infection. Relation to symptom formation and host defense. *Journal of Clinical Investigation*, *101*(3), 643–649. https://doi.org/10.1172/JCI1355

Hayney, M. S., Coe, C. L., Muller, D., Obasi, C. N., Backonja, U., Ewers, T., & Barrett, B. (2014). Age and psychological influences on immune responses to trivalent inactivated influenza vaccine in the meditation or exercise for preventing acute respiratory infection (MEPARI) trial. *Human Vaccines and Immunotherapeutics*, *10*(1), 83–91. https://doi.org/10.4161/hv.26661

Helwig, B. G., Craig, R. A., Fels, R. J., Blecha, F., & Kenney, M. J. (2008). Central nervous system administration of interleukin-6 produces splenic sympathoexcitation. *Autonomic Neuroscience*, *141*(1–2), 104–111. https://doi.org/10.1016/j.autneu.2008.04.008

Herbert, T. B., & Cohen, S. (1993). Depression and immunity: A meta-analytic review. *Psychological Bulletin*, *113*(3), 472–486. https://doi.org/10.1037/0033-2909.113.3.472

Hernandez, S., Vander Wal, J. S., & Spring, B. (2003). A negative mood induction procedure with efficacy across repeated administrations in women. *Journal of Psychopathology and Behavioral Assessment*, *25*(1), 49–55. https://doi.org/10.1023/A:1022252020196

Heron, M. (2007). Deaths: leading causes for 2004. *National Vital Statistics Reports : From the Centers for Disease Control and Prevention, National Center for Health Statistics, National Vital Statistics System*, *56*(5), 1–95. http://www.ncbi.nlm.nih.gov/pubmed/18092547

Herrman, H., Patrick, D. L., Diehr, P., Martin, M. L., Fleck, M., Simon, G. E., & Buesching, D. P. (2002). Longitudinal investigation of depression outcomes in primary care in six outcomes: The LIDO Study. Functional status, health service use and treatment of people with depressive symptoms. *Psychological Medicine*, *32*(5), 889–902. https://doi.org/10.1017/S003329170200586X

Hobson, D., Curry, R. L., Beare, A. S., & Ward-Gardner, A. (1972). The role of serum haemagglutination-inhibiting antibody in protection against challenge infection with influenza A2 and B viruses. *Journal of Hygiene*, *70*(4), 767–777. https://doi.org/10.1017/S0022172400022610

Holt-Lunstad, J., Smith, T. B., Baker, M., Harris, T., & Stephenson, D. (2015). Loneliness and Social Isolation as Risk Factors for Mortality: A Meta-Analytic Review. *Perspectives on Psychological Science*, *10*(2), 227–237. https://doi.org/10.1177/1745691614568352

Holtkamp, S. J., Ince, L. M., Barnoud, C., Schmitt, M. T., Sinturel, F., Pilorz, V., Pick, R., Jemelin, S., Mühlstädt, M., Boehncke, W.-H., Weber, J., Laubender, D., Philippou-Massier, J., Chen, C.-S., Holtermann, L., Vestweber, D., Sperandio, M., Schraml, B. U., Halin, C., … Scheiermann, C. (2021). Circadian clocks guide dendritic cells into skin lymphatics. *Nature Immunology*, *22*(11), 1375–1381. https://doi.org/10.1038/s41590-021-01040-x

Howell, R. T., Kern, M. L., & Lyubomirsky, S. (2007). Health benefits: Meta-analytically determining the impact of well-being on objective health outcomes. *Health Psychology Review*, *1*(1), 83–136. https://doi.org/10.1080/17437190701492486

Hsu, C. Y., Huang, L. M., Lee, C. Y., Lin, T. Y., Lee, P. I., & Chen, J. M. (1995). Local massage

after vaccination enhances the immunogenicity of diphtheria-tetanus-pertussis vaccine. *Pediatric Infectious Disease Journal*, *14*(7), 567–572. https://doi.org/10.1097/00006454-199507000-00003

Huang, F. Y., & Huang, L. M. (1999). Effect of local massage on vaccination: DTP and DTPa. *Acta Paediatrica Taiwanica = Taiwan Er Ke Yi Xue Hui Za Zhi*, *40*(3), 166–170. https://doi.org/10.7097/APS.199906.0166

Hucklebridge, F., Lambert, S., Clow, A., Warburton, D. M., Evans, P. D., & Sherwood, N. (2000). Modulation of secretory immunoglobulin A in saliva; response to manipulation of mood. *Biological Psychology*, *53*(1), 25–35. https://doi.org/10.1016/S0301-0511(00)00040-5

Irwin, M., Olmstead, R., & Oxman, M. (2007). Augmenting immune responses to varicella zoster virus in older adults: A randomized, controlled trial of Tai Chi. *Journal of the American Geriatrics Society*, *55*(4), 511–517. https://doi.org/10.1111/j.1532-5415.2007.01109.x

Irwin, M. R., & Slavich, G. M. (2016). Psychoneuroimmunology. In *Handbook of Psychophysiology* (pp. 377–398). Cambridge University Press. https://doi.org/10.1017/9781107415782.017

Jacobs, D. R., Adachi, H., Mulder, I., Kromhout, D., Menotti, A., Nissinen, A., & Blackburn, H. (1999). Cigarette smoking and mortality risk: Twenty-five-year follow-up of the Seven Countries Study. *Archives of Internal Medicine*, *159*(7), 733–740. https://doi.org/10.1001/archinte.159.7.733

Jacobsen, H., Rajendran, M., Choi, A., Sjursen, H., Brokstad, K. A., Cox, R. J., Palese, P., Krammer, F., & Nachbagauer, R. (2017). Influenza Virus Hemagglutinin Stalk-Specific Antibodies in Human Serum are a Surrogate Marker for In Vivo Protection in a Serum Transfer Mouse Challenge Model. *MBio*, *8*(5). https://doi.org/10.1128/mBio.01463-17

Janszky, I., Ericson, M., Lekander, M., Blom, M., Buhlin, K., Georgiades, A., & Ahnve, S. (2004). Inflammatory markers and heart rate variability in women with coronary heart disease. *Journal of Internal Medicine*, *256*(5), 421–428. https://doi.org/10.1111/j.1365-2796.2004.01403.x

Juslin, P. N., & Västfjäll, D. (2008). Emotional responses to music: The need to consider underlying mechanisms. *Behavioral and Brain Sciences*, *31*(5), 559–575. https://doi.org/10.1017/S0140525X08005293

Karin, O., Raz, M., Tendler, A., Bar, A., Korem Kohanim, Y., Milo, T., & Alon, U. (2020). A new model for the HPA axis explains dysregulation of stress hormones on the timescale of weeks. *Molecular Systems Biology*, *16*(7), e9510. https://doi.org/10.15252/msb.20209510

Kiecolt-Glaser, J. K., Belury, M. A., Porter, K., Beversdorf, D. Q., Lemeshow, S., & Glaser, R. (2007). Depressive symptoms, omega-6:omega-3 fatty acids, and inflammation in older adults. *Psychosomatic Medicine*, *69*(3), 217–224. https://doi.org/10.1097/PSY.0b013e3180313a45

Kiecolt-Glaser, J. K., Glaser, R., Gravenstein, S., Malarkey, W. B., & Sheridan, J. (1996). Chronic stress alters the immune response to influenza virus vaccine in older adults. *Proceedings of the National Academy of Sciences of the United States of America*, *93*(7), 3043–3047. https://doi.org/10.1073/pnas.93.7.3043

Kiecolt-Glaser, J. K., Graham, J. E., Malarkey, W. B., Porter, K., Lemeshow, S., & Glaser, R. (2008). Olfactory influences on mood and autonomic, endocrine, and immune function. *Psychoneuroendocrinology*, *33*(3), 328–339. https://doi.org/10.1016/j.psyneuen.2007.11.015

Kiecolt-Glaser, J. K., McGuire, L., Robles, T. F., & Glaser, R. (2002). Emotions, Morbidity, and Mortality: New Perspectives from Psychoneuroimmunology. *Annual Review of Psychology*, *53*(1), 83–107. https://doi.org/10.1146/annurev.psych.53.100901.135217

Kim, H., Webster, R. G., & Webby, R. J. (2018). Influenza Virus: Dealing with a Drifting and Shifting Pathogen. *Viral Immunology*, *31*(2), 174–183. https://doi.org/10.1089/vim.2017.0141

Kimata, H. (2004). Differential effects of laughter on allergen-specific immunoglobulin and neurotrophin levels in tears. *Perceptual and Motor Skills*, *98*(3 I), 901–908. https://doi.org/10.2466/pms.98.3.901-908

King, D. W., & Lashley, R. (2000). A quantifiable alternative to double data entry. *Controlled Clinical Trials*, *21*(2), 94–102. https://doi.org/10.1016/S0197-2456(00)00042-8

Kirchenbaum, G. A., Sautto, G. A., Richardson, R. A., Ecker, J. W., & Ross, T. M. (2021). A Competitive Hemagglutination Inhibition Assay for Dissecting Functional Antibody Activity against Influenza Virus. *Journal of Virology*, *95*(23). https://doi.org/10.1128/jvi.02379-20

Kirkpatrick, E., Qiu, X., Wilson, P. C., Bahl, J., & Krammer, F. (2018). The influenza virus hemagglutinin head evolves faster than the stalk domain. *Scientific Reports*, *8*(1), 1–14. https://doi.org/10.1038/s41598-018-28706-1

Kohut, M. L., Cooper, M. M., Nickolaus, M. S., Russell, D. R., & Cunnick, J. E. (2002). Exercise and psychosocial factors modulate immunity to influenza vaccine in elderly individuals. *Journals of Gerontology - Series A Biological Sciences and Medical Sciences*, *57*(9). https://doi.org/10.1093/gerona/57.9.M557

Kontou, E., Thomas, S., & Lincoln, N. (2012). Psychometric properties of a revised version of the Visual Analog Mood Scales. *Clinical Rehabilitation*, *26*(12), 1133–1140. https://doi.org/10.1177/0269215512442670

Koyama, M., Wachi, M., Utsuyama, M., Bittman, B., Hirokawa, K., & Kitagawa, M. (2009). Recreational music-making modulates immunological responses and mood states in older adults. *Journal of Medical and Dental Sciences*, *56*(2), 79–90. https://doi.org/10.11480/jmds.560201

Krakauer, T. (1995). Levels of interleukin 6 and tumor necrosis factor in serum from humans vaccinated with live, attenuated Francisella tularensis. *Clinical and Diagnostic Laboratory Immunology*, *2*(4), 487–488. https://doi.org/10.1128/cdli.2.4.487-488.1995

Krammer, F. (2019). The human antibody response to influenza A virus infection and vaccination. *Nature Reviews Immunology*, *19*(6), 383–397. https://doi.org/10.1038/s41577-019-0143-6

Kreutz, G., Ott, U., Teichmann, D., Osawa, P., & Vaitl, D. (2008). Using music to induce emotions: Influences of musical preference and absorption. *Psychology of Music*, *36*(1), 101–126. https://doi.org/10.1177/0305735607082623

Kubzansky, L. D., & Thurston, R. C. (2007). Emotional vitality and incident coronary heart

disease: Benefits of healthy psychological functioning. *Archives of General Psychiatry*, *64*(12), 1393–1401. https://doi.org/10.1001/archpsyc.64.12.1393

Kucuksezer, U. C., Aktas Cetin, E., Esen, F., Tahrali, I., Akdeniz, N., Gelmez, M. Y., & Deniz, G. (2021). The Role of Natural Killer Cells in Autoimmune Diseases. *Frontiers in Immunology*, *12*, 79. https://doi.org/10.3389/FIMMU.2021.622306/BIBTEX

Labott, S. M., Ahleman, S., Wolever, M. E., & Martin, R. B. (1990). The physiological and psychological effects of the expression and inhibition of emotion. *Behavioral Medicine*, *16*(4), 182–189. https://doi.org/10.1080/08964289.1990.9934608

Lacy, P., & Stow, J. L. (2011). Cytokine release from innate immune cells: association with diverse membrane trafficking pathways. *Blood*, *118*(1), 9–18. https://doi.org/10.1182/blood-2010-08-265892

Laidlaw, T. M., Booth, R. J., & Large, R. G. (1996). Reduction in skin reactions to histamine after a hypnotic procedure. *Psychosomatic Medicine*, *58*(3), 242–248. https://doi.org/10.1097/00006842-199605000-00008

Larsen, J. T., & McGraw, A. P. (2011). Further evidence for mixed emotions. *Journal of Personality and Social Psychology*, *100*(6), 1095–1110. https://doi.org/10.1037/a0021846

Lau, K. S., Partridge, E. A., Grigorian, A., Silvescu, C. I., Reinhold, V. N., Demetriou, M., & Dennis, J. W. (2007). Complex N-Glycan Number and Degree of Branching Cooperate to Regulate Cell Proliferation and Differentiation. *Cell*, *129*(1), 123–134. https://doi.org/10.1016/j.cell.2007.01.049

Laver, W. G., Air, G. M., Webster, R. G., & Smith-Gill, S. J. (1990). Epitopes on protein antigens: Misconceptions and realities. *Cell*, *61*(4), 553–556. https://doi.org/10.1016/0092-8674(90)90464-P

Lee, E. H. (2012). Review of the psychometric evidence of the perceived stress scale. *Asian Nursing Research*, *6*(4), 121–127. https://doi.org/10.1016/j.anr.2012.08.004

Lee, S. W., Youn, J. W., Seong, B. L., & Sung, Y. C. (1999). IL-6 induces long-term protective immunity against a lethal challenge of influenza virus. *Vaccine*, *17*(5), 490–496. https://doi.org/10.1016/S0264-410X(98)00223-0

Lewer, D., McKee, M., Gasparrini, A., Reeves, A., & de Oliveira, C. (2017). Socioeconomic position and mortality risk of smoking: evidence from the English Longitudinal Study of Ageing (ELSA). *European Journal of Public Health*, *27*(6), 1068–1073. https://doi.org/10.1093/eurpub/ckx059

Lewnard, J., & Cobey, S. (2018). Immune History and Influenza Vaccine Effectiveness. *Vaccines*, *6*(2), 28. https://doi.org/10.3390/vaccines6020028

Li, Y., Chen, S., Zhang, X., Fu, Q., Zhang, Z., Shi, S., Zhu, Y., Gu, M., Peng, D., & Liu, X. (2014). A 20-Amino-Acid Deletion in the Neuraminidase Stalk and a Five-Amino-Acid Deletion in the NS1 Protein Both Contribute to the Pathogenicity of H5N1 Avian Influenza Viruses in Mallard Ducks. *PLoS ONE*, *9*(4), e95539. https://doi.org/10.1371/journal.pone.0095539

Ligthart, G. J., Corberand, J. X., Fournier, C., Galanaud, P., Hijmans, W., Kennes, B., Müller-Hermelink, H. K., & Steinmann, G. G. (1984). Admission criteria for immunogerontological studies in man: The senieur protocol. *Mechanisms of Ageing and Development*, *28*(1), 47–55. https://doi.org/10.1016/0047-6374(84)90152-0

Lindhiem, O., Bennett, C. B., Trentacosta, C. J., & McLear, C. (2014). Client preferences affect treatment satisfaction, completion, and clinical outcome: A meta-analysis. *Clinical Psychology Review*, *34*(6), 506–517. https://doi.org/10.1016/j.cpr.2014.06.002

Loft, P., Petrie, K. J., Booth, R. J., Thomas, M. G., Robinson, E., & Vedhara, K. (2012). Effects of massage on antibody responses after hepatitis B vaccination. *Psychosomatic Medicine*, *74*(9), 982–987. https://doi.org/10.1097/PSY.0b013e31826fb7d2

Long, J. E., Drayson, M. T., Taylor, A. E., Toellner, K. M., Lord, J. M., & Phillips, A. C. (2016). Morning vaccination enhances antibody response over afternoon vaccination: A cluster-randomised trial. *Vaccine*, *34*(24), 2679–2685. https://doi.org/10.1016/j.vaccine.2016.04.032

Longo, D., & Koehn, K. (1993). Psychosocial factors and recurrent genital herpes: A review of prediction and psychiatric treatment studies. *International Journal of Psychiatry in Medicine*, *23*(2), 99–117. https://doi.org/10.2190/L5MH-0TCW-1PKD-5BM0

Lopez-Candales, A., Hernández Burgos, P. M., Hernandez-Suarez, D. F., & Harris, D. (2017). Linking Chronic Inflammation with Cardiovascular Disease: From Normal Aging to the Metabolic Syndrome. *Journal of Nature and Science*, *3*(4). /pmc/articles/PMC5488800/

Lozano, R., Naghavi, M., Foreman, K., Lim, S., Shibuya, K., Aboyans, V., Abraham, J., Adair, T., Aggarwal, R., Ahn, S. Y., AlMazroa, M. A., Alvarado, M., Anderson, H. R., Anderson, L. M., Andrews, K. G., Atkinson, C., Baddour, L. M., Barker-Collo, S., Bartels, D. H., … Murray, C. J. L. (2012). Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. *The Lancet*, *380*(9859), 2095–2128. https://doi.org/10.1016/S0140-6736(12)61728-0

Lutgendorf, S. K., Reimer, T. T., Harvey, J. H., Marks, G., Hong, S.-Y., Hillis, S. L., & Lubaroff, D. M. (2001). Effects of Housing Relocation on Immunocompetence and Psychosocial Functioning in Older Adults. *The Journals of Gerontology Series A: Biological Sciences and Medical Sciences*, *56*(2), M97–M105. https://doi.org/10.1093/gerona/56.2.M97

Ma, Q., Liu, J., Liu, Q., Kang, L., Liu, R., Jing, W., Wu, Y., & Liu, M. (2021). Global Percentage of Asymptomatic SARS-CoV-2 Infections Among the Tested Population and Individuals With Confirmed COVID-19 Diagnosis: A Systematic Review and Meta-analysis. *JAMA Network Open*, *4*(12), e2137257–e2137257. https://doi.org/10.1001/JAMANETWORKOPEN.2021.37257

Maciosek, M. V., Solberg, L. I., Coffield, A. B., Edwards, N. M., & Goodman, M. J. (2006). Influenza Vaccination. *American Journal of Preventive Medicine*, *31*(1), 72–79. https://doi.org/10.1016/j.amepre.2006.03.008

Maes, M., Meltzer, H. Y., Stevens, W., Calabrese, J., & Cosyns, P. (1994). Natural killer cell activity in major depression: Relation to circulating natural killer cells, cellular indices of the immune response, and depressive phenomenology. *Progress in Neuropsychopharmacology and Biological Psychiatry*, *18*(4), 717–730. https://doi.org/10.1016/0278-5846(94)90079-5

Maestroni, G. J. M. (2002). Short exposure of maturing, bone marrow-derived dendritic cells to norepinephrine: Impact on kinetics of cytokine production and Th development. *Journal of Neuroimmunology*, *129*(1–2), 106–114. https://doi.org/10.1016/S0165-5728(02)00188-1

Maestroni, G. J. M., & Mazzola, P. (2003). Langerhans cells β2-adrenoceptors: Role in migration, cytokine production, Th priming and contact hypersensitivity. *Journal of Neuroimmunology*, *144*(1–2), 91–99. https://doi.org/10.1016/j.jneuroim.2003.08.039

Manni, M., Granstein, R. D., & Maestroni, G. (2011). β2-Adrenergic agonists bias TLR-2 and NOD2 activated dendritic cells towards inducing an IL-17 immune response. *Cytokine*, *55*(3), 380–386. https://doi.org/10.1016/j.cyto.2011.05.013

Marques-Deak, A., Cizza, G., & Sternberg, E. (2005). Brain-immune interactions and disease susceptibility. *Molecular Psychiatry*, *10*(3), 239–250. https://doi.org/10.1038/sj.mp.4001643

Marsland, A. L., Cohen, S., Rabin, B. S., & Manuck, S. B. (2001). Associations between stress, trait negative affect, acute immune reactivity, and antibody response to hepatitis B injection in healthy young adults. *Health Psychology*, *20*(1), 4–11. https://doi.org/10.1037/0278-6133.20.1.4

Marsland, A. L., Cohen, S., Rabin, B. S., & Manuck, S. B. (2006). Trait positive affect and antibody response to hepatitis B vaccination. *Brain, Behavior, and Immunity*, *20*(3), 261–269. https://doi.org/10.1016/j.bbi.2005.08.009

Marsland, A. L., Pressman, S., & Cohen, S. (2007). Positive Affect and Immune Function. In *Psychoneuroimmunology* (Vol. 2, pp. 761–779). Elsevier. https://doi.org/10.1016/B978-012088576-3/50042-3

Maryam Fakhrhosseini, S., & Jeon, M. (2017). Affect/Emotion Induction Methods. In *Emotions and Affect in Human Factors and Human-Computer Interaction* (pp. 235–253). Elsevier. https://doi.org/10.1016/B978-0-12-801851-4.00010-0

Mastorakos, G., & Ilias, I. (2006). Interleukin-6: A Cytokine and/or a Major Modulator of the Response to Somatic Stress. *Annals of the New York Academy of Sciences*, *1088*(1), 373–381. https://doi.org/10.1196/annals.1366.021

Matsunaga, M., Bai, Y., Yamakawa, K., Toyama, A., Kashiwagi, M., Fukuda, K., Oshida, A., Sanada, K., Fukuyama, S., Shinoda, J., Yamada, J., Sadato, N., & Ohira, H. (2013). Brain-Immune Interaction Accompanying Odor-Evoked Autobiographic Memory. *PLoS ONE*, *8*(8), e72523. https://doi.org/10.1371/journal.pone.0072523

Matsunaga, M., Isowa, T., Kimura, K., Miyakoshi, M., Kanayama, N., Murakami, H., Sato, S., Konagaya, T., Nogimori, T., Fukuyama, S., Shinoda, J., Yamada, J., & Ohira, H. (2008). Associations among central nervous, endocrine, and immune activities when positive emotions are elicited by looking at a favorite person. *Brain, Behavior, and Immunity*, *22*(3), 408–417. https://doi.org/10.1016/j.bbi.2007.09.008

Matsunaga, M., Isowa, T., Yamakawa, K., Kawanishi, Y., Tsuboi, H., Kaneko, H., Sadato, N., Oshida, A., Katayama, A., Kashiwagi, M., & Ohira, H. (2011). Psychological and physiological responses to odor-evoked autobiographic memory. *Activitas Nervosa Superior Rediviva*, *32*(6), 774–780.

McAuley, J. L., Gilbertson, B. P., Trifkovic, S., Brown, L. E., & McKimm-Breschkin, J. L. (2019). Influenza Virus Neuraminidase Structure and Functions. *Frontiers in Microbiology*, *10*(JAN), 39. https://doi.org/10.3389/fmicb.2019.00039

McClelland, D. C., Floor, E., Davidson, R. J., & Saron, C. (1980). Stressed Power Motivation, Sympathetic Activation, Immune Function, and Illness. *Journal of Human Stress*, *6*(2), 11–19. https://doi.org/10.1080/0097840X.1980.9934531

McElhaney, J. E., Xie, D., Hager, W. D., Barry, M. B., Wang, Y., Kleppinger, A., Ewen, C., Kane, K. P., & Bleackley, R. C. (2006). T Cell Responses Are Better Correlates of Vaccine Protection in the Elderly. *The Journal of Immunology*, *176*(10), 6333–6339. https://doi.org/10.4049/jimmunol.176.10.6333

McEwen, B. S. (1998). Stress, adaptation, and disease. Allostasis and allostatic load. *Annals of the New York Academy of Sciences*, *840*(1), 33–44. https://doi.org/10.1111/j.1749-6632.1998.tb09546.x

McGill, C. J., & Benayoun, B. A. (2022). Time isn't kind to female T cells. *Nature Aging*, *2*(3), 189–191. https://doi.org/10.1038/s43587-022-00185-0

McKee, A. S., Munks, M. W., & Marrack, P. (2007). How Do Adjuvants Work? Important Considerations for New Generation Adjuvants. *Immunity*, *27*(5), 687–690. https://doi.org/10.1016/j.immuni.2007.11.003

Meyer, R. J., & Haggerty, R. J. (1962). Streptococcal infection in families. *Nursing Research*, *11*(2), 122. https://doi.org/10.1097/00006199-196201120-00100

Meyers, E., Heytens, S., Formukong, A., Vercruysse, H., De Sutter, A., Geens, T., Hofkens, K., Janssens, H., Nys, E., Padalko, E., Deschepper, E., & Cools, P. (2021). Comparison of Dried Blood Spots and Venous Blood for the Detection of SARS-CoV-2 Antibodies in a Population of Nursing Home Residents. *Microbiology Spectrum*, *9*(2). https://doi.org/10.1128/Spectrum.00178-21

Middleton, R. A., & Byrd, E. K. (1996). Psychosocial Factors and Hospital Readmission Status of Older Persons with Cardiovascular Disease. *Journal of Applied Rehabilitation Counseling*, *27*(4), 3–10. https://doi.org/10.1891/0047-2220.27.4.3

Mittwoch-Jaffe, T., Shalit, F., Srendi, B., & Yehuda, S. (1995). Modification of cytokine secretion following mild emotional stimuli. *NeuroReport*, *6*(5), 789–792. https://doi.org/10.1097/00001756-199503270-00021

Monto, A. S., Ohmit, S. E., Petrie, J. G., Johnson, E., Truscon, R., Teich, E., Rotthoff, J., Boulton, M., & Victor, J. C. (2009). Comparative Efficacy of Inactivated and Live Attenuated Influenza Vaccines. *New England Journal of Medicine*, *361*(13), 1260–1267. https://doi.org/10.1056/NEJMoa0808652

Monto, A. S., & Petrie, J. G. (2019). Improving Influenza Vaccine Effectiveness: Ways to Begin Solving the Problem. *Clinical Infectious Diseases*, *69*(10), 1824–1826. https://doi.org/10.1093/cid/ciz416

Moreno-Smith, M., Lutgendorf, S. K., & Sood, A. K. (2010). Impact of stress on cancer metastasis. *Future Oncology*, *6*(12), 1863–1881. https://doi.org/10.2217/fon.10.142

Morgan, R., Gao, G., Pawling, J., Dennis, J. W., Demetriou, M., & Li, B. (2004). N -Acetylglucosaminyltransferase V (Mgat5)-Mediated N -Glycosylation Negatively Regulates Th1 Cytokine Production by T Cells. *The Journal of Immunology*, *173*(12), 7200–7208. https://doi.org/10.4049/jimmunol.173.12.7200

Morrison, S. F. (2011). 2010 Carl Ludwig Distinguished Lectureship of the APS Neural Control and Autonomic Regulation Section: Central neural pathways for thermoregulatory cold defense. *Journal of Applied Physiology*, *110*(5), 1137–1149. https://doi.org/10.1152/japplphysiol.01227.2010

Moynihan, J. A., Larson, M. R., Treanor, J., Duberstein, P. R., Power, A., Shore, B., & Ader, R. (2004). Psychosocial Factors and the Response to Influenza Vaccination in Older

Adults. *Psychosomatic Medicine*, *66*(6), 950–953. https://doi.org/10.1097/01.psy.0000140001.49208.2d

Müller, C., Dubiel, D., Kremeti, E., Lieb, M., Streicher, E., Siakir Oglou, N., Mickel, C., & Karbach, J. (2021). Effects of a Single Physical or Mindfulness Intervention on Mood, Attention, and Executive Functions: Results from two Randomized Controlled Studies in University Classes. *Mindfulness*, *12*(5), 1282–1293. https://doi.org/10.1007/s12671-021-01601-z

Murasko, D. M., Weiner, P., & Kaye, D. (1987). Decline in mitogen induced proliferation of lymphocytes with increasing age. *Clinical and Experimental Immunology (Print)*, *70*(2), 440–448.

Murialdo, G., Barreca, A., Nobili, F., Rollero, A., Timossi, G., Gianelli, M. V., Copello, F., Rodriguez, G., & Polleri, A. (2001). Relationships between cortisol, dehydroepiandrosterone sulphate and insulin-like growth factor-I system in dementia. *Journal of Endocrinological Investigation*, *24*(3), 139–146. https://doi.org/10.1007/BF03343833

Murphy, E., Smith, R., Lindesay, J., & Slattery, J. (1988). Increased Mortality Rates in Late-life Depression. *British Journal of Psychiatry*, *152*(3), 347–353. https://doi.org/10.1192/bjp.152.3.347

National Institute for Health and Care Excellence. (2018). *Resource impact report: Flu vaccination: Increasing uptake (NG103)*.

Nes, L. S., & Segerstrom, S. C. (2017). Psychoneuroimmunology☆. In *Reference Module in Neuroscience and Biobehavioral Psychology*. Elsevier. https://doi.org/10.1016/B978-0-12-809324-5.22186-X

NICE. (2019). *INFLUENZA VACCINE | Medicinal forms | BNF content published by NICE*. https://bnf.nice.org.uk/medicinal-forms/influenza-vaccine.html

Nieman, D. C., Henson, D. A., Austin, M. D., & Brown, V. A. (2005). Immune response to a 30-minute walk. *Medicine and Science in Sports and Exercise*, *37*(1), 57–62. https://doi.org/10.1249/01.MSS.0000149808.38194.21

Nieman, D. C., & Wentz, L. M. (2019). The compelling link between physical activity and the body's defense system. *Journal of Sport and Health Science*, *8*(3), 201–217. https://doi.org/10.1016/j.jshs.2018.09.009

Njus, D. M., Nitschke, W., & Bryant, F. B. (1996). Positive affect, negative affect, and the moderating effect of writing on sIgA antibody levels. *Psychology and Health*, *12*(1), 135–148. https://doi.org/10.1080/08870449608406927

Noto, Y., Kitajima, M., Kudo, M., Okudera, K., & Hirota, K. (2010). Leg massage therapy promotes psychological relaxation and reinforces the first-line host defense in cancer patients. *Journal of Anesthesia*, *24*(6), 827–831. https://doi.org/10.1007/s00540-010-1028-9

Novack, D. H., Cameron, O., Epel, E., Ader, R., Waldstein, S. R., Levenstein, S., Antoni, M. H., & Wainer, A. R. (2007). Psychosomatic Medicine: The Scientific Foundation of the Biopsychosocial Model. *Academic Psychiatry*, *31*(5), 388–401. https://doi.org/10.1176/appi.ap.31.5.388

Novello, F., Ridolfi, B., Fiore, L., Buttinelli, G., Medda, E., Favero, A., Marchetti, D., & Gaglioppa, F. (1996). Comparison of capillary blood versus venous blood samples in

the assessment of immunity to measles. *Journal of Virological Methods*, *61*(1–2), 73–77. https://doi.org/10.1016/0166-0934(96)02071-X

O'Garra, A. (1998). Cytokines Induce the Development of Functionally Heterogeneous T Helper Cell Subsets. *Immunity*, *8*(3), 275–283. https://doi.org/10.1016/S1074-7613(00)80533-6

Office for National Statistics. (2020). *Leading causes of death, UK: 2001 to 2018. Registered leading causes of death by age, sex and country.* Leading Causes of Death, UK: 2001 to 2018. https://www.ons.gov.uk/peoplepopulationandcommunity/healthandsocialcare/causesofdeath/articles/leadingcausesofdeathuk/2001to2018

Oshitani, H., Kamigaki, T., & Suzuki, A. (2008). Major Issues and Challenges of Influenza Pandemic Preparedness in Developing Countries. *Emerging Infectious Diseases*, *14*(6), 875–880. https://doi.org/10.3201/eid1406.070839

Ostir, G. V., Markides, K. S., Peek, M. K., & Goodwin, J. S. (2001). The association between emotional well-being and the incidence of stroke in older adults. *Psychosomatic Medicine*, *63*(2), 210–215. https://doi.org/10.1097/00006842-200103000-00003

Oxford Vaccine Group. (2018). *Influenza (flu)*. Vaccine Knowledge [Online]. http://vk.ovg.ox.ac.uk/influenza-flu

Paget, J., Spreeuwenberg, P., Charu, V., Taylor, R. J., Iuliano, A. D., Bresee, J., Simonsen, L., & Viboud, C. (2019). Global mortality associated with seasonal influenza epidemics: New burden estimates and predictors from the GLaMOR Project. *Journal of Global Health*, *9*(2). https://doi.org/10.7189/jogh.09.020421

Papanicolaou, D. A., Wilder, R. L., Manolagas, S. C., & Chrousos, G. P. (1998). The pathophysiologic roles of interleukin-6 in human disease. *Annals of Internal Medicine*, *128*(2), 127–137. https://doi.org/10.7326/0003-4819-128-2-199801150-00009

Parens, H., McConville, B. J., & Kaplan, S. M. (1966). The Prediction of Frequency of Illness from the Response to Seperation. *Psychosomatic Medicine*, *28*(2), 162–176. https://doi.org/10.1097/00006842-196603000-00006

Parikh, S. R., Andrews, N. J., Beebeejaun, K., Campbell, H., Ribeiro, S., Ward, C., White, J. M., Borrow, R., Ramsay, M. E., & Ladhani, S. N. (2016). Effectiveness and impact of a reduced infant schedule of 4CMenB vaccine against group B meningococcal disease in England: a national observational cohort study. *The Lancet*, *388*(10061), 2775–2782. https://doi.org/10.1016/S0140-6736(16)31921-3

Pascoe, A. R., Fiatarone Singh, M. A., & Edwards, K. M. (2014). The effects of exercise on vaccination responses: A review of chronic and acute exercise interventions in humans. *Brain, Behavior, and Immunity*, *39*, 33–41. https://doi.org/10.1016/j.bbi.2013.10.003

Patrozou, E., & Mermel, L. A. (2009). Does Influenza Transmission Occur from Asymptomatic Infection or Prior to Symptom Onset? *Public Health Reports*, *124*(2), 193–196. https://doi.org/10.1177/003335490912400205

Pawelec, G. (2018). Age and immunity: What is "immunosenescence"? *Experimental Gerontology*, *105*, 4–9. https://doi.org/10.1016/j.exger.2017.10.024

Pedersen, A. F., Zachariae, R., & Bovbjerg, D. H. (2009). Psychological stress and antibody response to influenza vaccination: A meta-analysis. *Brain, Behavior, and Immunity*,

*23*(4), 427–433. https://doi.org/10.1016/j.bbi.2009.01.004

Pereira, B., Xu, X.-N., & Akbar, A. N. (2020). Targeting Inflammation and Immunosenescence to Improve Vaccine Responses in the Elderly. *Frontiers in Immunology*, *11*. https://doi.org/10.3389/fimmu.2020.583019

Petrie, K. J., Booth, R. J., Pennebaker, J. W., Davison, K. P., et al, & Thomas, M. (1995). Disclosure of trauma and immune response to a hepatitis B vaccination program. *Journal of Consulting and Clinical Psychology*, *63*(5), 787–792. https://doi.org/10.1037/0022-006X.63.5.787

Petrova, V. N., & Russell, C. A. (2018). The evolution of seasonal influenza viruses. *Nature Reviews Microbiology*, *16*(1), 47–60. https://doi.org/10.1038/nrmicro.2017.118

Petrovsky, N., & Harrison, L. C. (1998). The chronobiology of human cytokine production. *International Reviews of Immunology*, *16*(5–6), 635–649. https://doi.org/10.3109/08830189809043012

Phillips, A. C., Burns, V. E., Carroll, D., Ring, C., & Drayson, M. (2005). The association between life events, social support, and antibody status following thymus-dependent and thymus-independent vaccinations in healthy young adults. *Brain, Behavior, and Immunity*, *19*(4), 325–333. https://doi.org/10.1016/J.BBI.2004.10.004

Phillips, A. C., Carroll, D., Burns, V. E., Ring, C., Macleod, J., & Drayson, M. (2006). Bereavement and marriage are associated with antibody response to influenza vaccination in the elderly. *Brain, Behavior, and Immunity*, *20*(3), 279–289. https://doi.org/10.1016/j.bbi.2005.08.003

Plotkin, S. A. (2010). Correlates of protection induced by vaccination. *Clinical and Vaccine Immunology*, *17*(7), 1055–1065. https://doi.org/10.1128/CVI.00131-10

Pongratz, G., & Straub, R. H. (2014). The sympathetic nervous response in inflammation. *Arthritis Research and Therapy*, *504*(16). https://doi.org/10.1186/s13075-014-0504-2

Posner, J., Russell, J. A., & Peterson, B. S. (2005). The circumplex model of affect: An integrative approach to affective neuroscience, cognitive development, and psychopathology. *Development and Psychopathology*, *17*(3), 715–734. https://doi.org/10.1017/S0954579405050340

Powis, S. (2019). *Vaccines for 2019/20 seasonal flu vaccination program*. https://bnf.nice.org.uk/medicinal-forms/influenza-vaccine.html.

Prather, A. A., Hall, M., Fury, J. M., Ross, D. C., Muldoon, M. F., Cohen, S., & Marsland, A. L. (2012). Sleep and Antibody Response to Hepatitis B Vaccination. *SLEEP*. https://doi.org/10.5665/sleep.1990

Prather, A. A., Pressman, S. D., Miller, G. E., & Cohen, S. (2021). Temporal Links Between Self-Reported Sleep and Antibody Responses to the Influenza Vaccine. *International Journal of Behavioral Medicine*, *28*(1), 151–158. https://doi.org/10.1007/s12529-020-09879-4

Pressman, S. D., & Cohen, S. (2005). Does positive affect influence health? *Psychological Bulletin*, *131*(6), 925–971. https://doi.org/10.1037/0033-2909.131.6.925

Principi, N., & Esposito, S. (2012). Adjuvanted influenza vaccines. *Human Vaccines & Immunotherapeutics*, *8*(1), 59–66. https://doi.org/10.4161/hv.8.1.18011

Public Health England. (2014). *Public Health England and the NHS prepare for unpredictable*

*flu season*. https://www.gov.uk/government/news/public-health-england-and-the-nhs-prepare-for-unpredictable-flu-season

Public Health England. (2018). *Seasonal Influenza Vaccination Annual Report 2017/18: South West*. https://www.england.nhs.uk/south/wp-content/uploads/sites/6/2018/09/phe-sw-flu-review-2017-18.pdf

Raber, J. (1998). Detrimental effects of chronic hypothalamic-pituitary-adrenal axis activation: From obesity to memory deficits. *Molecular Neurobiology*, *18*(1), 1–22. https://doi.org/10.1007/BF02741457

Ramirez, A. J., Craig, T. K. J., Watson, J. P., Fentiman, I. S., North, W. R. S., & Rubens, R. D. (1989). Stress and relapse of breast cancer. *British Medical Journal*, *298*(6669), 291–293. https://doi.org/10.1136/bmj.298.6669.291

Reiche, E. M. V., Morimoto, H. K., & Nunes, S. O. V. (2005). Stress and depression-induced immune dysfunction: Implications for the development and progression of cancer. *International Review of Psychiatry*, *17*(6), 515–527. https://doi.org/10.1080/02646830500382102

Reis, H. T., Sheldon, K. M., Gable, S. L., Roscoe, J., & Ryan, R. M. (2000). Daily well-being: The role of autonomy, competence, and relatedness. *Personality and Social Psychology Bulletin*, *26*(4), 419–435. https://doi.org/10.1177/0146167200266002

Remarque, E. J., de Bruijn, I. A., Boersma, W. J. A., Masurel, N., & Ligthart, G. J. (1998). Altered antibody response to influenza H1N1 vaccine in healthy elderly people as determined by HI, ELISA, and neutralization assay. *Journal of Medical Virology*, *55*(1), 82–87. https://doi.org/10.1002/(SICI)1096-9071(199805)55:1<82::AID-JMV13>3.0.CO;2-1

Remington, N. A., Fabrigar, L. R., & Visser, P. S. (2000). Reexamining the circumplex model of affect. *Journal of Personality and Social Psychology*, *79*(2), 286–300. https://doi.org/10.1037/0022-3514.79.2.286

Rider, M. S., Achterberg, J., Lawlis, G. F., Goven, A., Toledo, R., & Butler, J. R. (1990). Effect of immune system imagery on secretory IgA. *Biofeedback and Self-Regulation*, *15*(4), 317–333. https://doi.org/10.1007/BF01000026

Riepl, K., Mussel, P., Osinsky, R., & Hewig, J. (2016). Influences of State and Trait Affect on Behavior, Feedback-Related Negativity, and P3b in the Ultimatum Game. *PLOS ONE*, *11*(1), e0146358. https://doi.org/10.1371/journal.pone.0146358

Robles, T. F., Brooks, K. P., & Pressman, S. D. (2009). Trait Positive Affect Buffers the Effects of Acute Stress on Skin Barrier Recovery. *Health Psychology*, *28*(3), 373–378. https://doi.org/10.1037/a0014662

Robles, T. F., Glaser, R., & Kiecolt-Glaser, J. K. (2005). Out of Balance. *Current Directions in Psychological Science*, *14*(2), 111–115. https://doi.org/10.1111/j.0963-7214.2005.00345.x

Rose, J. P., Geers, A. L., Fowler, S. L., & Rasinski, H. M. (2014). Choice-making, Expectations, and Treatment Positivity: How and When Choosing Shapes Aversive Experiences. *Journal of Behavioral Decision Making*, *27*(1), 1–10. https://doi.org/10.1002/bdm.1775

Rosenkranz, M. A., Jackson, D. C., Dalton, K. M., Dolski, I., Ryff, C. D., Singer, B. H., Muller, D., Kalin, N. H., & Davidson, R. J. (2003). Affective style and in vivo immune response:

Neurobehavioral mechanisms. *Proceedings of the National Academy of Sciences of the United States of America*, *100*(19), 11148–11152. https://doi.org/10.1073/pnas.1534743100

Roszman, T. L., Jackson, J. C., Cross, R. J., Titus, M. J., Markesbery, W. R., & Brooks, W. H. (1985). Neuroanatomic and neurotransmitter influences on immune function. *Journal of Immunology (Baltimore, Md. : 1950)*, *135*(2 Suppl), 769s-772s. http://www.ncbi.nlm.nih.gov/pubmed/2861232

Ryff, C. D., & Singer, B. (2003). Ironies of the human condition: Well-being and health on the way to mortality. In *A psychology of human strengths: Fundamental questions and future directions for a positive psychology.* (pp. 271–287). American Psychological Association. https://doi.org/10.1037/10566-019

Ryff, C. D., Singer, B. H., & Dienberg Love, G. (2004). Positive health: connecting well–being with biology. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences*, *359*(1449), 1383–1394. https://doi.org/10.1098/rstb.2004.1521

Sacadura-Leite, E., Sousa-Uva, A., Rebelo-De-Andrade, H., Ferreira, S., & Rocha, R. (2014). Association between chronic stress and immune response to influenza vaccine in healthcare workers. *Revista Portuguesa de Saude Publica*, *32*(1), 18–26. https://doi.org/10.1016/j.rpsp.2013.09.002

Salovey, P., & Birnbaum, D. (1989). Influence of mood on health-relevant cognitions. *Journal of Personality and Social Psychology*, *57*(3), 539–551. https://doi.org/10.1037/0022-3514.57.3.539

Salovey, P., Rothman, A. J., Detweiler, J. B., & Steward, W. T. (2000). Emotional states and physical health. *American Psychologist*, *55*(1), 110–121. https://doi.org/10.1037/0003-066X.55.1.110

Sarason, I. G., Sarason, B. R., Potter, E. H., & Antoni, M. H. (1985). Life Events, Social Support, and Illness. *Psychosomatic Medicine*, *47*(2), 156–163. https://doi.org/10.1097/00006842-198503000-00007

Sasaki, S., He, X.-S., Holmes, T. H., Dekker, C. L., Kemble, G. W., Arvin, A. M., & Greenberg, H. B. (2008). Influence of Prior Influenza Vaccination on Antibody and B-Cell Responses. *PLoS ONE*, *3*(8), e2975. https://doi.org/10.1371/journal.pone.0002975

Schleifer, S. J., Keller, S. E., Bartlett, J. A., Eckholdt, H. M., & Delaney, B. R. (1996). Immunity in young adults with major depressive disorder. *American Journal of Psychiatry*, *153*(4), 477–482. https://doi.org/10.1176/ajp.153.4.477

Schmukle, S. C., Egloff, B., & Burns, L. R. (2002). The relationship between positive and negative affect in the Positive and Negative Affect Schedule. *Journal of Research in Personality*, *36*(5), 463–475. https://doi.org/10.1016/S0092-6566(02)00007-7

Schneiderman, N., Antoni, M. H., Saab, P. G., & Ironson, G. (2001). Health Psychology: Psychosocial and Biobehavioral Aspects of Chronic Disease Management. *Annual Review of Psychology*, *52*(1), 555–580. https://doi.org/10.1146/annurev.psych.52.1.555

Schneiderman, N., Ironson, G., & Siegel, S. D. (2005). Stress and Health: Psychological, Behavioral, and Biological Determinants. *Annual Review of Clinical Psychology*, *1*(1), 607–628. https://doi.org/10.1146/annurev.clinpsy.1.102803.144141

Schoenborn, J. R., & Wilson, C. B. (2007). *Regulation of Interferon-γ During Innate and*

*Adaptive Immune Responses* (pp. 41–101). https://doi.org/10.1016/S0065-2776(07)96002-2

Segerstrom, S. C., Hardy, J. K., Evans, D. R., & Greenberg, R. N. (2012). Vulnerability, distress, and immune response to vaccination in older adults. *Brain, Behavior, and Immunity*, *26*(5), 747–753. https://doi.org/10.1016/j.bbi.2011.10.009

Segerstrom, S. C., & Miller, G. E. (2004). Psychological Stress and the Human Immune System: A Meta-Analytic Study of 30 Years of Inquiry. *Psychological Bulletin*, *130*(4), 601–630. https://doi.org/10.1037/0033-2909.130.4.601

Segerstrom, S. C., Schipper, L. J., & Greenberg, R. N. (2008). Caregiving, repetitive thought, and immune response to vaccination in older adults. *Brain, Behavior, and Immunity*, *22*(5), 744–752. https://doi.org/10.1016/j.bbi.2007.11.004

Segerstrom, S. C., Taylor, S. E., Kemeny, M. E., & Fahey, J. L. (1998). Optimism is associated with mood, coping, and immune change in response to stress. *Journal of Personality and Social Psychology*, *74*(6), 1646–1655. https://doi.org/10.1037/0022-3514.74.6.1646

Seiffert, K., Hosoi, J., Torii, H., Ozawa, H., Ding, W., Campton, K., Wagner, J. A., & Granstein, R. D. (2002). Catecholamines Inhibit the Antigen-Presenting Capability of Epidermal Langerhans Cells. *The Journal of Immunology*, *168*(12), 6128–6135. https://doi.org/10.4049/jimmunol.168.12.6128

Seifter, E., Rettura, G., Zisblatt, M., Levenson, S. M., Levine, N., Davidson, A., & Seifter, J. (1973). Enhancement of tumor development in physically-stressed mice inoculated with an oncogenic virus. *Experientia*, *29*(11), 1379–1382. https://doi.org/10.1007/BF01922831

Sekhon, M., Cartwright, M., & Francis, J. J. (2017). Acceptability of healthcare interventions: An overview of reviews and development of a theoretical framework. *BMC Health Services Research*, *17*(1), 1–13. https://doi.org/10.1186/s12913-017-2031-8

Shekarchi, I. C., Sever, J. L., Tzan, N., Ley, A., Ward, L. C., & Madden, D. (1981). Comparison of hemagglutination inhibition test and enzyme-linked immunosorbent assay for determining antibody to rubella virus. *Journal of Clinical Microbiology*, *13*(5), 850–854. https://doi.org/10.1128/jcm.13.5.850-854.1981

Sheldon, K. M., Ryan, R., & Reis, H. T. (1996). What makes for a good day? Competence and autonomy in the day and in the person. *Personality and Social Psychology Bulletin*, *22*(12), 1270–1279. https://doi.org/10.1177/01461672962212007

Sheng, J. A., Bales, N. J., Myers, S. A., Bautista, A. I., Roueinfar, M., Hale, T. M., & Handa, R. J. (2021). The Hypothalamic-Pituitary-Adrenal Axis: Development, Programming Actions of Hormones, and Maternal-Fetal Interactions. *Frontiers in Behavioral Neuroscience*, *14*, 256. https://doi.org/10.3389/fnbeh.2020.601939

Sicca, F., Martinuzzi, D., Montomoli, E., & Huckriede, A. (2020). Comparison of influenza-specific neutralizing antibody titers determined using different assay readouts and hemagglutination inhibition titers: good correlation but poor agreement. *Vaccine*, *38*(11), 2527–2541. https://doi.org/10.1016/j.vaccine.2020.01.088

Siegrist, C. (2013). Section 1: General Aspects of Vaccination. Part 2. Vaccine Immunology. In *Vaccines* (6th ed., pp. 17–36). Elsevier. http://www.sciencedirect.com/science/book/9781455700905#ancsc0010

Smith, K., Machalaba, C. C., Seifman, R., Feferholtz, Y., & Karesh, W. B. (2019). Infectious disease and economics: The case for considering multi-sectoral impacts. *One Health*, *7*, 100080. https://doi.org/10.1016/j.onehlt.2018.100080

Smith, S. M., & Vale, W. W. (2006). The role of the hypothalamic-pituitary-adrenal axis in neuroendocrine responses to stress. *Dialogues in Clinical Neuroscience*, *8*(4), 383–395. https://doi.org/10.31887/DCNS.2006.8.4/ssmith

Solana, R., & Pawelec, G. (2004). *Immunosenescence* (pp. 9–21). https://doi.org/10.1016/S1567-7443(04)80003-6

Solana, R., Tarazona, R., Gayoso, I., Lesur, O., Dupuis, G., & Fulop, T. (2012). Innate immunosenescence: Effect of aging on cells and receptors of the innate immune system in humans. *Seminars in Immunology*, *24*(5), 331–341. https://doi.org/10.1016/j.smim.2012.04.008

Solerte, S. B., Fioravanti, M., Vignati, G., Giustina, A., Cravello, L., & Ferrari, E. (1999). Dehydroepiandrosterone Sulfate Enhances Natural Killer Cell Cytotoxicity in Humans Via Locally Generated Immunoreactive Insulin-Like Growth Factor I 1. *The Journal of Clinical Endocrinology & Metabolism*, *84*(9), 3260–3267. https://doi.org/10.1210/jcem.84.9.6003

Spengler, R. N., Allen, R. M., Remick, D. G., Strieter, R. M., & Kunkel, S. L. (1990). Stimulation of alpha-adrenergic receptor augments the production of macrophage-derived tumor necrosis factor. *Journal of Immunology (Baltimore, Md. : 1950)*, *145*(5), 1430–1434. http://www.ncbi.nlm.nih.gov/pubmed/2166759

Stech, O., Veits, J., Abdelwhab, E.-S. M., Wessels, U., Mettenleiter, T. C., & Stech, J. (2015). The Neuraminidase Stalk Deletion Serves as Major Virulence Determinant of H5N1 Highly Pathogenic Avian Influenza Viruses in Chicken. *Scientific Reports*, *5*(1), 13493. https://doi.org/10.1038/srep13493

Steptoe, A., Dockray, S., & Wardle, J. (2009). Positive affect and psychobiological processes relevant to health. *Journal of Personality*, *77*(6), 1747–1776. https://doi.org/10.1111/j.1467-6494.2009.00599.x

Steptoe, A., & Kivimäki, M. (2013). Stress and Cardiovascular Disease: An Update on Current Knowledge. *Annual Review of Public Health*, *34*(1), 337–354. https://doi.org/10.1146/annurev-publhealth-031912-114452

Steptoe, A., O'Donnell, K., Badrick, E., Kumari, M., & Marmot, M. (2008). Neuroendocrine and inflammatory factors associated with positive affect in healthy men and women: The Whitehall II study. *American Journal of Epidemiology*, *167*(1), 96–102. https://doi.org/10.1093/aje/kwm252

Steptoe, A., Owen, N., Kunz-Ebrecht, S., & Mohamed-Ali, V. (2002). Inflammatory cytokines, socioeconomic status, and acute stress responsivity. *Brain, Behavior, and Immunity*, *16*(6), 774–784. https://doi.org/10.1016/S0889-1591(02)00030-2

Sternberg, E. M. (2006). Neural regulation of innate immunity: A coordinated nonspecific host response to pathogens. *Nature Reviews Immunology*, *6*(4), 318–328.

Sternberg, Esther M., Glowa, J. R., Smith, M. A., Cologero, A. E., Listwak, S. J., Aksentijevich, S., Chrousos, G. P., Wilder, R. L., & Gold, P. W. (1992). Corticotropin releasing hormone related behavioral and neuroendocrine responses to stress in Lewis and Fischer rats. *Brain Research*, *570*(1–2), 54–60. https://doi.org/10.1016/0006-

8993(92)90563-O

Stetler, C., Chen, E., & Miller, G. E. (2006). Written disclosure of experiences with racial discrimination and antibody response to an influenza vaccine. *International Journal of Behavioral Medicine*, *13*(1), 60–68. https://doi.org/10.1207/s15327558ijbm1301_8

Stone, A. A., Cox, D. S., Valdimarsdottir, H., Jandorf, L., & Neale, J. M. (1987). Evidence That Secretory IgA Antibody Is Associated With Daily Mood. *Journal of Personality and Social Psychology*, *52*(5), 988–993. https://doi.org/10.1037/0022-3514.52.5.988

Stone, A. A., Marco, C. A., Cruise, C. E., Cox, D. S., & Neale, J. M. (1996). Are stress-induced immunological changes mediated by mood? A closer look at how both desirable and undesirable daily events influence sIgA antibody. *International Journal of Behavioral Medicine*, *3*(1), 1–13. https://doi.org/10.1207/s15327558ijbm0301_1

Stone, A. A., Neale, J. M., Cox, D. S., Napoli, A., Valdimarsdottir, H., & Kennedy-Moore, E. (1994). Daily Events Are Associated With a Secretory Immune Response to an Oral Antigen in Men. *Health Psychology*, *13*(5), 440–446. https://doi.org/10.1037/0278-6133.13.5.440

Stone, A. A., Porter, L. S., & Neale, J. M. (1993). Daily events and mood prior to the onset of respiratory illness episodes: A non-replication of the 3–5 day 'desirability dip.' *British Journal of Medical Psychology*, *66*(4), 383–393. https://doi.org/10.1111/j.2044-8341.1993.tb01764.x

Stroud, L. R., Salovey, P., & Epel, E. S. (2002). Sex differences in stress responses: Social rejection versus achievement stress. *Biological Psychiatry*, *52*(4), 318–327. https://doi.org/10.1016/S0006-3223(02)01333-1

Tabue Teguo, M., Simo-Tabue, N., Stoykova, R., Meillon, C., Cogne, M., Amiéva, H., & Dartigues, J. F. (2016). Feelings of Loneliness and Living Alone as Predictors of Mortality in the Elderly: The PAQUID Study. *Psychosomatic Medicine*, *78*(8), 904–909. https://doi.org/10.1097/PSY.0000000000000386

Takahashi, K., Iwase, M., Yamashita, K., Tatsumoto, Y., Ue, H., Kuratsune, H., Shimizu, A., & Takeda, M. (2001). The elevation of natural killer cell activity induced by laughter in a crossover designed study. *International Journal of Molecular Medicine*, *8*(6), 645–650. https://doi.org/10.3892/ijmm.8.6.645

Tanaka, T., Narazaki, M., & Kishimoto, T. (2014). Il-6 in inflammation, Immunity, And disease. *Cold Spring Harbor Perspectives in Biology*, *6*(10), 16295–16296. https://doi.org/10.1101/cshperspect.a016295

Taylor, A. W., Wilson, D. H., Grande, E. D., Ben-Tovim, D., Elzinga, R. H., Goldney, R. D., MacFarlane, A. C., Cheok, F., & Kirke, K. (2000). Mental health status of the South Australian population. *Australian and New Zealand Journal of Public Health*, *24*(1), 29–34. https://doi.org/10.1111/j.1467-842X.2000.tb00719.x

Taylor, D. J., Kelly, K., Kohut, M. L., & Song, K. S. (2017). Is Insomnia a Risk Factor for Decreased Influenza Vaccine Response? *Behavioral Sleep Medicine*, *15*(4), 270–287. https://doi.org/10.1080/15402002.2015.1126596

Taylor, S. E., Klein, L. C., Lewis, B. P., Gruenewald, T. L., Gurung, R. A. R., & Updegraff, J. A. (2000). Biobehavioral responses to stress in females: Tend-and-befriend, not fight-or-flight. *Psychological Review*, *107*(3), 411–429. https://doi.org/10.1037/0033-295X.107.3.411

Teilegen, A. (2019). Structures of Mood and Personality and Their Relevance to Assessing Anxiety, With an Emphasis on Self-Report. In *Anxiety and the Anxiety Disorders* (pp. 681–706). Routledge. https://doi.org/10.4324/9780203728215-49

Terry, D. J., & O'Leary, J. E. (1995). The theory of planned behaviour: The effects of perceived behavioural control and self-efficacy. *British Journal of Social Psychology*, *34*(2), 199–220. https://doi.org/10.1111/j.2044-8309.1995.tb01058.x

Thompson, E. R. (2007). Development and validation of an internationally reliable short-form of the Positive and Negative Affect Schedule (PANAS). *Journal of Cross-Cultural Psychology*, *38*(2), 227–242. https://doi.org/10.1177/0022022106297301

Thorpe, J. M., Sleath, B. L., Thorpe, C. T. K., Van Houtven, C. H., Blalock, S. J., Landerman, L. R., Campbell, W. H., & Clipp, E. C. (2006). Caregiver psychological distress as a barrier to influenza vaccination among community-dwelling elderly with dementia. *Medical Care*, *44*(8), 713–721. https://doi.org/10.1097/01.mlr.0000215905.36968.76

Tilvis, R. S., Laitala, V., Routasalo, P. E., & Pitkälä, K. H. (2011). Suffering from Loneliness Indicates Significant Mortality Risk of Older People. *Journal of Aging Research*, *2011*, 1–5. https://doi.org/10.4061/2011/534781

Tischner, D., & Reichardt, H. M. (2007). Glucocorticoids in the control of neuroinflammation. *Molecular and Cellular Endocrinology*, *275*(1–2), 62–70. https://doi.org/10.1016/J.MCE.2007.03.007

Topping, J., Reardon, M., Coleman, J., Hunter, B., Shojima-Perera, H., Thyer, L., & Simpson, P. (2019). A Comparison of Venous versus Capillary Blood Samples when Measuring Blood Glucose Using a Point-of-Care, Capillary-Based Glucometer. *Prehospital and Disaster Medicine*, *34*(5), 506–509. https://doi.org/10.1017/S1049023X19004850

Totman, R., Kiff, J., Reed, S. E., & Craig, J. W. (1980). Predicting experimental colds in volunteers from different measures of recent life stress. *Journal of Psychosomatic Research*, *24*(3–4), 155–163. https://doi.org/10.1016/0022-3999(80)90037-9

Tracey, K. J. (2007). Physiology and immunology of the cholinergic antiinflammatory pathway. *Journal of Clinical Investigation*, *117*(2), 289–296. https://doi.org/10.1172/JCI30555

Tracey, K. J. (2009). Reflex control of immunity. *Nature Reviews Immunology*, *9*(6), 418–428. https://doi.org/10.1038/nri2566

Treanor, J. (2004). Influenza Vaccine — Outmaneuvering Antigenic Shift and Drift. *New England Journal of Medicine*, *350*(3), 218–220. https://doi.org/10.1056/NEJMp038238

Tregoning, J. S., Russell, R. F., & Kinnear, E. (2018). Adjuvanted influenza vaccines. *Human Vaccines and Immunotherapeutics*, *14*(3), 550–564. https://doi.org/10.1080/21645515.2017.1415684

Trombetta, C. M., Remarque, E. J., Mortier, D., & Montomoli, E. (2018). Comparison of hemagglutination inhibition, single radial hemolysis, virus neutralization assays, and ELISA to detect antibody levels against seasonal influenza viruses. *Influenza and Other Respiratory Viruses*, *12*(6), 675–686. https://doi.org/10.1111/irv.12591

Trzonkowski, P., Myśliwska, J., Pawelec, G., & Myśliwski, A. (2009). From bench to bedside and back: The SENIEUR Protocol and the efficacy of influenza vaccination in the elderly. *Biogerontology*, *10*(1), 83–94. https://doi.org/10.1007/s10522-008-9155-5

Tsukamoto, H., Fujieda, K., Senju, S., Ikeda, T., Oshiumi, H., & Nishimura, Y. (2018). Immune-suppressive effects of interleukin-6 on T-cell-mediated anti-tumor immunity. *Cancer Science*, *109*(3), 523–530. https://doi.org/10.1111/cas.13433

Van Buynder, P. G., Konrad, S., Van Buynder, J. L., Brodkin, E., Krajden, M., Ramler, G., & Bigham, M. (2013). The comparative effectiveness of adjuvanted and unadjuvanted trivalent inactivated influenza vaccine (TIV) in the elderly. *Vaccine*, *31*(51), 6122–6128. https://doi.org/10.1016/j.vaccine.2013.07.059

van Doorn, H. R. (2014). Emerging infectious diseases. *Medicine*, *42*(1), 60–63. https://doi.org/10.1016/j.mpmed.2013.10.014

VanderPlate, C., Aral, S. O., & Magder, L. (1988). The relationship among genital herpes simplex virus, stress, and social support. *Health Psychology : Official Journal of the Division of Health Psychology, American Psychological Association*, *7*(2), 159–168. https://doi.org/10.1037/0278-6133.7.2.159

Vedhara, K., Ayling, K., Sunger, K., Caldwell, D. M., Halliday, V., Fairclough, L., Avery, A., Robles, L., Garibaldi, J., Welton, N. J., & Royal, S. (2019). Psychological interventions as vaccine adjuvants: A systematic review. *Vaccine*, *37*(25), 3255–3266. https://doi.org/10.1016/j.vaccine.2019.04.091

Vedhara, K., Bennett, P. D. P. D., Clark, S., Lightman, S. L. S. L., Shaw, S., Perks, P., Hunt, M. A. M. A., Philip, J. M. D. J. M. D., Tallon, D., P.J., M., R.W., J., G.K., W., N.M., S., Murphy, P. J., Jones, R. W., Wilcock, G. K., & Shanks, N. M. (2003). Enhancement of antibody responses to influenza vaccination in the elderly following a cognitive-behavioural stress management intervention. *Psychotherapy and Psychosomatics*, *72*(5), 245–252. https://doi.org/10.1159/000071895

Vedhara, K., Cox, N. K., Wilcock, G. K., Perks, P., Hunt, M., Anderson, S., Lightman, S. L., & Shanks, N. M. (1999). Chronic stress in elderly carers of dementia patients and antibody response to influenza vaccination. *The Lancet*, *353*(9153), 627–631. https://doi.org/10.1016/S0140-6736(98)06098-X

Veitch, J. A., & Newsham, G. R. (2000). Exercised control, lighting choices, and energy use: An office simulation experiment. *Journal of Environmental Psychology*, *20*(3), 219–237. https://doi.org/10.1006/jevp.1999.0169

Walsh, N. P., Gleeson, M., Shephard, R. J., Gleeson, M., Woods, J. A., Bishop, N. C., Fleshner, M., Green, C., Pedersen, B. K., Hoffman-Goetz, L., Rogers, C. J., Northoff, H., Abbasi, A., & Simon, P. (2011). Position statement. Part one: Immune function and exercise. *Exercise Immunology Review*, *17*, 6–63. /articles/journal_contribution/Position_statement_part_one_immune_function_and_exercise/9630083/1

Wan, H., Sultana, I., Couzens, L. K., Mindaye, S., & Eichelberger, M. C. (2017). Assessment of influenza A neuraminidase (subtype N1) potency by ELISA. *Journal of Virological Methods*, *244*, 23–28. https://doi.org/10.1016/j.jviromet.2017.02.015

Ware, J., Kosinski, M., & Keller, S. D. (1996). A 12-Item Short-Form Health Survey: Construction of Scales and Preliminary Tests of Reliability and Validity. *Medical Care*, *34*(3), 220–233. https://doi.org/10.1097/00005650-199603000-00003

Ware, J., Kosinski, M., & Keller, S. D. (1998). How to score the SF12 Physical and Mental Health Summary Scales. *The Health Institute, New England Medical Center, Boston*.

Ware, J., MA, K., Tuner-bowker, D., & Gandek, B. (2002). *How to score SF-12 items Estimation of medical care total expenditures*. https://www.researchgate.net/publication/291994160

Watson, D., & Clark, L. A. (1997). Measurement and Mismeasurement of Mood: Recurrent and Emergent issues. *Journal of Personality Assessment*, *68*(2), 267–296. https://doi.org/10.1207/s15327752jpa6802_4

Watson, D., Clark, L. A., & Carey, G. (1988). Positive and negative affectivity and their relation to anxiety and depressive disorders. *Journal of Abnormal Psychology*, *97*(3), 346–353. https://doi.org/10.1037/0021-843X.97.3.346

Watson, D., Clark, L. A., & Tellegen, A. (1988). Development and validation of brief measures of positive and negative affect: The PANAS scales. *Journal of Personality and Social Psychology*, *54*(6), 1063–1070. https://doi.org/10.1037/0022-3514.54.6.1063

Waxenbaum, J. A., Reddy, V., & Varacallo, M. (2022). Anatomy, Autonomic Nervous System. In *StatPearls*. http://www.ncbi.nlm.nih.gov/pubmed/30969667

Whiteside, T. L., Bryant, J., Herberman, R. B., & Day, R. (1990). Natural killer cytotoxicity in the diagnosis of immune dysfunction: Criteria for a reproducible assay. *Journal of Clinical Laboratory Analysis*, *4*(2), 102–114. https://doi.org/10.1002/jcla.1860040207

Wilson, N. J., Boniface, K., Chan, J. R., McKenzie, B. S., Blumenschein, W. M., Mattson, J. D., Basham, B., Smith, K., Chen, T., Morel, F., Lecron, J. C., Kastelein, R. A., Cua, D. J., McClanahan, T. K., Bowman, E. P., & Malefyt, R. de W. (2007). Development, cytokine profile and function of human interleukin 17-producing helper T cells. *Nature Immunology*, *8*(9), 950–957. https://doi.org/10.1038/ni1497

Wong, S. Y. S., Wong, C. K., Chan, F. W. K., Chan, P. K. S., Ngai, K., Mercer, S., & Woo, J. (2013). Chronic psychosocial stress: does it modulate immunity to the influenza vaccine in Hong Kong Chinese elderly caregivers? *AGE*, *35*(4), 1479–1493. https://doi.org/10.1007/s11357-012-9449-z

World Health Organisation. (2011). *Manual for the Laboratory Diagnosis and Virological Surveillance of Influenza*. http://apps.who.int/iris/bitstream/handle/10665/44518/9789241548090_eng.pdf;jsessionid=7FA4E0C846E7D4BA8ACBD3D4F44B51CF?sequence=1

World Health Organisation. (2015). *Vaccine effectiveness estimates for seasonal influenza vaccines*. https://www.who.int/influenza/vaccines/virus/recommendations/201502_qanda_vaccineeffectiveness.pdf

World Health Organisation. (2018). *Influenza (Seasonal)*. https://www.who.int/en/news-room/fact-sheets/detail/influenza-(seasonal)

World Health Organisation. (2019). *Biologicals: Influenza*. https://www.who.int/biologicals/areas/vaccines/influenza/en/

Wrona, D. (2006). Neural-immune interactions: An integrative view of the bidirectional relationship between the brain and immune systems. *Journal of Neuroimmunology*, *172*(1–2), 38–58. https://doi.org/10.1016/j.jneuroim.2005.10.017

Wurster, A. L., & Grusby, M. J. (2004). Cytokines. In *Encyclopedia of Biological Chemistry* (pp. 550–555). Elsevier. https://doi.org/10.1016/B0-12-443710-9/00298-2

Yancy, W. S., Mayer, S. B., Coffman, C. J., Smith, V. A., Kolotkin, R. L., Geiselman, P. J., McVay, M. A., Oddone, E. Z., & Voils, C. I. (2015). Effect of allowing choice of diet on weight loss: A randomized trial. *Annals of Internal Medicine*, *162*(12), 805–814. https://doi.org/10.7326/M14-2358

Yang, J., Zhang, J., Han, T., Liu, C., Li, X., Yan, L., Yang, B., & Yang, X. (2020). Effectiveness, immunogenicity, and safety of influenza vaccines with MF59 adjuvant in healthy people of different age groups. *Medicine*, *99*(7), e19095. https://doi.org/10.1097/MD.0000000000019095

Yang, Y., Verkuilen, J., Rosengren, K. S., Mariani, R. A., Reed, M., Grubisich, S. A., Woods, J. A., & Schlagal, B. (2008). Effects of a Traditional Taiji/Qigong Curriculum on Older Adults'Immune Response to Influenza Vaccine. In *Tai Chi Chuan* (Vol. 52, pp. 64–76). KARGER. https://doi.org/10.1159/000134285

Yu, M., & Clark, M. (2015). Investigating Mindfulness, Borderline Personality Traits, and Well-Being in a Nonclinical Population. *Psychology*, *06*(10), 1232–1248. https://doi.org/10.4236/psych.2015.610121

Zhao, X., Siegel, K., Chen, M. I. C., & Cook, A. R. (2017). Rethinking thresholds for serological evidence of influenza virus infection. *Influenza and Other Respiratory Viruses*, *11*(3), 202–210. https://doi.org/10.1111/irv.12452

Zimmermann, P., Ritz, N., Perrett, K. P., Messina, N. L., van der Klis, F. R. M., & Curtis, N. (2021). Correlation of Vaccine Responses. *Frontiers in Immunology*, *12*, 869. https://doi.org/10.3389/fimmu.2021.646677

Zorrilla, E. P., Luborsky, L., McKay, J. R., Rosenthal, R., Houldin, A., Tax, A., McCorkle, R., Seligman, D. A., & Schmidt, K. (2001). The relationship of depression and stressors to immunological assays: A meta-analytic review. *Brain, Behavior, and Immunity*, *15*(3), 199–226. https://doi.org/10.1006/brbi.2000.0597

# Appendix A

Appendix A- Ethical approval letter.

# Appendix B

Appendix B- Participant information sheet.

# Appendix C

Appendix C- Participant invitation sheet and reply slip.

# Appendix D

Appendix D- Baseline Questionnaire (Demographic Information, Perceived Stress, Trait Affect and Health Status).

Part A: Demographic Information.

Part B: Health Status (Physical and Mental Health, SF-12).

Part C- Positive and Negative Affect Scale- Short Form (PANAS-SF).

Part D- Perceived Stress Scale (PSS).

# Appendix E

Appendix E- Consent form.

# Appendix F

Appendix F- State affect measures (SPANE, Affective Slider and DVAMS) pre- and post-intervention.

**Scale of Positive and Negative Experience (SPANE)** (Diener et al., 2009)

You will now be shown some words that people use to describe different feelings and emotions. Read each word and select the option that best reflects the extent to which you feel that way right now. When you are ready to move on, press continue.

| 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|
| Very rarely or never | Rarely | Sometimes | Often | Very often or always |

Positive, Negative, Good, Bad, Pleasant, Unpleasant, Happy, Sad, Afraid, Joyful, Angry, Contented.

**Affective Slider** (Betella & Verschure, 2016)

On the next page you will be presented with two sliders like the one below. The pictures on each end represents the top and bottom of a scale. Use your finger to slide/drag the circle to mark where on the scale you think best represents how you feel right now. You can practice using the slider below. When you are ready to move on, press continue.

1. Mark on the slider below how **Happy** you are right now:

2. Mark on the slider below how **Alert** you are right now:

**Dynamic Visual Analogue Mood Scales (D-VAMS)** (Barrows & Thomas, 2016)

You are about to see more sliders, similar to the ones you just completed. However, next to these sliders there is an image of a face, whose expression you can modify using the slider control on the right. Move the slider up and down until the face has an expression that best reflects how you are feeling right now. You will now have an opportunity to practice this on the next page. When you are ready to move on, press continue.