

**PSYCHOSOCIAL FACTORS, PHYSICAL ACTIVITY STATUS AND ANTIBODY  
RESPONSE TO VACCINATION IN HEALTHY AND HIV POSITIVE  
POPULATIONS**

**By**

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## ABSTRACT

This thesis examines the effects of psychosocial factors and physical activity on antibody response to vaccination in healthy young, older, and HIV+ populations. Chapter Two found that a brisk walk prior to vaccination did not improve antibody response to pneumococcal or influenza vaccinations in young (18-30yrs) or older (50-64yrs) adults. Chapter Three examined whether a lifestyle physical activity intervention affected antibody response to pneumococcal vaccination in sedentary middle-aged women. There was no effect on antibody response, body composition or fitness measures, although there was an improvement in quality of life for the intervention group. Finally, Chapter Four investigated the relationship between psychosocial and physical activity status and antibody response to vaccination in HIV+ patients. Antibody response to some strains of the pneumococcal vaccine were predicted by higher physical activity levels (pn1, pn6b, pn18c), greater social support (pn3) and lower life events stress (pn1). However, the majority of analyses found that antibody response to vaccination was not affected by these measures. In conclusion, neither acute nor chronic walking interventions improve antibody response to vaccination, and only limited relationships are seen between psychosocial factors, physical activity status and antibody response to a variety of vaccinations.

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## LIST OF PAPERS

This thesis incorporates the following two papers:

- Long, J.E., Ring, C., Drayson, M.T., Bosch, J.A., Campbell, J.P., Bhabra, J., Browne, D., Dawson J., Harding, S., Lau, J., Burns., V.E. Vaccination response following aerobic exercise: Can a brisk walk enhance antibody response to pneumococcal and influenza vaccinations? *Brain, Behaviour and Immunity*, *in press*.
- Long, J.E., Ring, C., Drayson, M.T., Bosch, J.A., Eves, F., Allen, D., Calver, R., Say, V., Burns., V.E. A lifestyle physical activity intervention and the antibody response to pneumococcal vaccination in women, *in preparation*.

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- Long, J.E., Burns, V.E., Ring, C., Bosch, J.A., Drayson, M.T., Browne, D., Dawson, J., Harding, S., Lau, J., Bhabra, J., The effect of an acute aerobic exercise intervention on antibody response to pneumococcal and influenza vaccination. *International Society of Exercise Immunology meeting*, 2011.\*

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- Edwards, K.M., Campbell, J.P., Ring, C., Drayson, M.T., Bosch, J.A., Downes, C., Long, J.E., Lumb, J.A., Merry, A., Paine, N.J., Burns, V.E., 2010. Exercise intensity does not influence the efficacy of eccentric exercise as a behavioural adjuvant to vaccination. *Brain, Behaviour and Immunity* 24, 623-30.

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## CHAPTER ONE

### GENERAL INTRODUCTION

The effects of psychological stress and exercise on immune function, such as an increased risk of upper respiratory tract infection, are well documented (Solomon, 1962; Meyer and Haggerty, 1962; Neiman, 1994). Following on from these early studies of naturalistic infection, research focused on identifying which aspects of immune function may be altered by these behavioural influences (Ader, 1981; Gleeson, 2006). For example, enumerative and *in vitro* functional measures have previously been examined as measures of immune function. However, these measures cannot demonstrate cell function or take into account the complex interactions within the immune environment. One of the most theoretically robust methods of examining *in vivo* immune function is the vaccination model (Burns and Gallagher, 2010), as it generates a cascade of immune process which ultimately produce antibodies as a quantifiable measurement of the response. This chapter will introduce the vaccination model and discuss of the evidence that psychosocial factors and exercise can influence the antibody response to vaccination in healthy young, older, and HIV+ populations.

#### **The vaccination model**

The vaccination model can be used in two principle ways, either as a quantitative measure of vaccine efficacy or as a general marker of immune function. Previous studies have used exogenous immunological adjuvants, which are substances added to the vaccine that help induce more immunity than the antigen alone (Ramon, 1924). However, studies have also

given some attention to interventions which induce putative endogenous adjuvants, such as cytokines or other danger signals (Edwards and Campbell, 2011). Vaccines can also be used as a more general marker of immunity to examine how well the immune system responds to a specific pathogen. For example, exposure and timing can be controlled by administering a standard vaccination dose, whereas naturalistic exposure to disease cannot control for this. Both models are particularly useful when examining immunocompromised populations, such as HIV+ patients, as they tend to suffer from an increased risk of infections. Therefore, it is important to examine methods to enhance the efficacy of the antibody response to ensure adequate protection from the vaccinated disease, and also as a general marker to determine whether immune function is affected by behavioural influence.

### **Antibody response following vaccination**

One way the immune system responds to pathogenic challenge is by producing antibodies specific to the antigen. Following vaccination with a novel protein antigen, such as influenza, keyhole limpet hemocyanin (KLH) or hepatitis B, dendritic cells ingest and process the antigen to form a peptide fragment, which is presented on a major histocompatibility (MHC) II molecule on the cell's membrane. These dendritic cells move from the tissues to the lymph nodes, via the lymphatic system, to present the membrane bound antigen to a CD4+ T helper (Th) cell. The CD4+ T cell receptor CD28 binds with CD80 and CD86 receptors on the antigen presenting cell, which provides co-stimulation of the T cell response. At the same time, B cells specific to the antigen bind to any free antigen in the tissue or lymph, and travel to the lymph node. The stimulated CD4+ cell then binds to the B cell displaying the antigen on its membrane (Abbas and Lichtman, 2006). This activates the B cell to produce

antibodies, which opsonise and neutralise the antigen and help eliminate infection. Once infection has been removed, some T and B lymphocytes specific to the antigen remain in the lymph nodes as memory cells. These cells activate and proliferate rapidly upon re-exposure, and produce a more specific antibody with a stronger affinity to the antigen. The immune response to non-protein antigens, such as polysaccharides, does not require T cell activation in order to combat infection, and thus are considered thymus independent. In these cases, polysaccharide antigens bind directly to B cells which differentiate into antibody secreting plasma cells. The multivalency of T independent antigens induces immunoglobulin cross linking on the cell's surface membrane, which enhances B cell stimulation (Mond et al, 1979; 1995). Therefore, relatively low levels of antigen concentration are needed in order to stimulate a B cell response (Brunswick et al, 1988). Both T dependent and independent responses produce antigen-specific antibodies which can be measured in serum as a marker of the extent of the vaccination response. Not only as previous research has indicated that both types of vaccinations are susceptible to behavioural influences (Edwards et al, 2008; Burns et al, 2003; Burns and Gallagher, 2010), but it is important to examine both types of vaccination. For example, depending on the nature of the antigen of the vaccine, it can stimulate different aspects of the immune system, such as the T dependent or independent pathway; this could help elucidate which aspects of immunity are susceptible to behavioural influence.

## **ACUTE STRESS AND EXERCISE**

### **Animal literature**

Research examining the cell mediated and humoral immune response to vaccination initially focussed on psychological stress interventions, and to a lesser extent, acute exercise. Psychological stress paradigms in rodents have involved restraint, tail shock, foot shock and shaking procedure, designed to impose a stressful, but not dangerous, situation. In 1996, Dhabhar and McEwen examined the delayed-type hypersensitivity (DTH) response, which is an antigen-specific, cell-mediated immune response (Dhabhar and McEwen, 1999), to 2, 4-dinitrofluorobenzene (DNFB) in rats experiencing restraint stress. They found that acute restraint stress induced a significant enhancement of the delayed-type hypersensitivity (DTH) response and when the severity of stress was increased, by including shaking, a further increase in the DTH response was seen; this was not apparent when just the duration of the stressor was increased (Dhabhar and McEwen, 1997). This enhancement of the DTH response was mirrored by increased leukocyte redeployment from the blood to organs such as the skin and lymph nodes. Therefore, this migration of lymphocytes to the site of immune challenge was proposed to mediate the effect of stress on immune function.

Although Dhabhar and colleagues focussed on the cell mediated response following vaccination, other studies have investigated the humoral response to vaccination. Millan et al (1996) found that the rats which had been restrained for 2 hours per day for 2 days after inoculation with sheep red blood cells (SRBCs) showed improved antibody titres after 7 days, compared to the rats with no restraint stress. Similarly, Wood et al (1993) found that foot shock stress on the two days prior to keyhole limpet cyanine (KLH) vaccination augmented anti-KLH IgG levels in rats. In addition, Silberman et al (2003) found that rats which were

immunized with SRBCs following 2 hours of restraint stress and then received a booster vaccination on day 11 had elevated anti-SRBC IgG titres at 18 day follow up, compared to the control condition. Therefore, this suggests that both primary and secondary immune response in rodents is augmented by psychological stress. Karp and colleagues (2000) found that mice treated with cyclophosphamide, an immunosuppressant drug, which underwent restraint stress had higher IgM and IgG antibody titres following KLH administration at 8, 14 and 21 days post vaccination, compared to non-restrained immunosuppressed mice. Furthermore, restrained, immunosuppressed mice also had a better IgG antibody response compared to restrained mice, which were not immunosuppressed; this suggests an interaction where there was a greater effect of the restraint intervention for the immunosuppressed mice. Kapasi et al (2000) investigated the effect of a single intense bout of exercise prior to human serum albumin (HSA) vaccine booster in young and aged rats. Results showed that after 9 days, the exercised older rats showed augmented HSA antibody responses, compared to the control mice, whereas the young rats showed no difference in antibody titre. This literature again suggests that a psychological stress and exercise intervention may be more beneficial when immune responses tend to be poor, and robust immune responses are less likely to be affected by stress exposure. Although there is some controversy in this literature, with some laboratories reporting either no effect or a negative effect of acute stress on antibody response to vaccination (Fleshner et al, 1995; 1998; Gazda et al, 2003; Laudenslager et al, 1988), recent controlled trials suggest that there is a much more consistent positive effect in humans.

## **Human literature**

Previous naturalistic studies examined the effects of competitive races on antibody responses and found conflicting results (Eskola et al, 1978; Bruunsgaard et al, 1997). However, these were non-randomised, underpowered trials which only looked at highly trained participants. Edwards et al (2006a) was the first randomised controlled trial to examine whether an acute stressor would enhance antibody response to influenza vaccination. This study found that a mental arithmetic task and an exercise cycling protocol significantly enhanced the antibody response to one of the three influenza strains in female participants. The authors also found that the meningococcal antibody responses were higher in men, but not women, who had carried out the exercise intervention (Edwards et al, 2008). The results indicated that the intervention only improved antibody titres when the control group had a poor response. Edwards et al (2006b) showed that IL-6 was positively associated with antibody response at 60 minutes post exercise and 4 and 20 weeks post vaccination, which suggests IL-6 may be an underlying mechanism between exercise and improved antibody response. This is plausible as one study found that successful responders to a live vaccine were characterised by higher circulating IL-6 levels, compared to non-responders (Krakauer, 1995). A more localised stressor, eccentric exercise, has shown increases in ‘danger signals’, such as IL-6 levels and creatine kinase (Hirose et al, 2004; Willoughby et al, 2003), which may act as natural adjuvants to activate dendritic cells (Gallucci and Matzinger, 2001; Gallucci et al, 1999; Proske and Morgan, 2001). Edwards et al (2007), therefore, used eccentric resistance exercise of the biceps brachii and deltoid muscles, and found enhanced antibody response to one of the influenza strains in women, and the cell-mediated IFN- $\gamma$  response in men. Again, effects were only seen in less immunogenic strains which elicited a poor response to the vaccination

in the control group; this implies a ceiling effect may have occurred where robust antibody responses were not further improved by the intervention.

As with animal studies (Wood et al, 1993; Millan et al, 1996), the timing of these factors may be important considering the rise in various cytokines at different time points following the exercise. Campbell et al (2009) investigated the timing of antibody response to vaccination in relation to eccentric exercise; participants were vaccinated immediately following exercise during muscle oedema, 6 hours post exercise at the time of peak IL-6 response, and 48 hours post exercise to coincide with the point of heightened inflammatory environment and clearance of muscle damage. However, results showed that all participants had robust responses to the vaccination, eccentric exercise did not further augment antibody response, and there were no differences between the groups. This implies that it is only when the control group's response to vaccination is poor that eccentric exercise has an effect upon antibody response.

Edwards et al (2010) varied the intensity of eccentric exercise prior to a half dose influenza vaccine and found that the exercise intervention was effective in augmenting antibody response to two of the three influenza strains, compared to the control group. However, there was no relationship between the weight used (60%, 85%, or 110% of the individual's one repetition maximum) and subsequent antibody response (Edwards et al, 2010). To account for any ceiling effects of the vaccination, a half dose influenza vaccination was used to mimic a poor antibody response to vaccination. These results suggest that populations with reduced antibody responses to vaccination, such as older adults (Grubeck-Loebenstien et al, 2009), may benefit more from an exercise intervention, compared to more robust populations.

In recent years, eccentric exercise has experienced a significant amount of attention in the context of its potential as an adjuvant for vaccination response. However, the effects of aerobic exercise have received much less consideration. As the intensity of exercise does not appear to be related to the magnitude of the antibody response within eccentric exercise (Edwards et al, 2010), it is possible that an acute moderate intensity aerobic exercise intervention, such as a brisk walk, may have similar benefits to antibody response. This type of moderate intensity exercise intervention may be more suitable and easier to implement within a clinical population, such as the elderly or immunocompromised.



## **CHRONIC EXERCISE AND ANTIBODY RESPONSE TO VACCINATION**

The effects of chronic exposure to exercise and stress can also be examined using the vaccine model. In this case, the antibody response to vaccination is used as a marker of general immune function, rather than as a marker of vaccine efficacy, with a poorer antibody response indicating a generally lower ability to respond effectively to an antigen. This section will outline the studies which have investigated long-term exercise or stress and antibody response to vaccination in animals and humans.

### **Exercise training in animals**

Liu and Wang (1987) was one of the first studies to investigate a chronic training intervention upon antibody response in rodents. They found that mice which ran for 10 minutes twice a day for 18 weeks had higher antibody responses to a second dose of *Salmonella typhi*, compared to the mice which remained sedentary. Furthermore, Kaufman et al (1994) found that secondary IgM, and to a lesser extent IgG, antibody response to a KLH booster vaccination was enhanced in rats undertaking a graded moderate intensity swim exercise for 4 weeks. These two early studies suggest that a structured training intervention can enhance antibody response to vaccination.

Voluntary wheel running protocols, in which animals are caged either with or without a running wheel, have also been used in some studies as a less structured exercise intervention. Suzuki and Tagami (2005) found that rats housed with a running wheel for 32 days showed an increase in tetanus toxoid IgG response six days following tetanus toxoid booster, compared to rats housed without a running wheel. In contrast, Rogers et al (2008) found that mice with access to a running wheel for 8 weeks showed no differences in antibody response following

vaccination, compared to mice caged with a running wheel. However, in this study average running distance was 5.5km/day, whereas in the final week of the Suzuki and Tagami (2005) study, rats had a mean running distance of 8.2km/day; it is possible that improvement in antibody response is intensity dependent.

Although some evidence suggests that a moderate intensity physical activity intervention does improve antibody response to vaccination in rodents, few studies have tried to elucidate the mechanisms behind this. Kapasi et al (2001) found that antibody responses to a human serum albumin booster vaccine in exercising and non-exercising mice, which had received naltrexone (opioid antagonist) implantation, placebo or control, were poorer in the exercising naltrexone group, compared to the other exercising non-naltrexone group. This proposes that endogenous opioids may play an important role in the relationship between exercise and secondary antibody response. Furthermore, Kohut et al (2004a) found that adrenergic blockade decreased the exercise induced improvements, which were seen in anti-HSV IgM and IL-2 and IFN- $\gamma$  cytokines following an 8 week training protocol in old mice. This suggests that the effects of chronic exercise could be mediated by  $\beta$  adrenergic receptors, which are stimulated by the increase in catecholamines following chronic exercise. Therefore, endogenous opioids or  $\beta$  adrenergic receptors may be possible mechanisms through which exercise improves antibody response.

Although most studies have found a positive relationship between chronic exercise training and some aspects of immune function, there are a few studies which have failed to find a relationship with antibody response following vaccination in rodents (Wang et al, 2011; Rogers et al, 2008; Barnes et al, 1991; Coleman and Rager, 1994). However, this may be due to the habitual voluntary exercise programme which was used (Rogers et al, 2008), or that a primary vaccination is less susceptible to the effects of physical activity (Barnes et al, 1991;

Coleman and Rager, 1994). Nevertheless, as the majority of research suggests that chronic exercise interventions may be beneficial for vaccination response, particularly within an aged population, this provides a meaningful rationale for researchers to investigate the role of exercise and antibody response to vaccination in humans.

### **Exercise training in humans**

Nieman (1994) described a 'J shaped' curve relationship between exercise intensity and immune function, which indicated that those who engaged in moderate levels of physical activity had fewer upper respiratory tract infections (URTI) compared to those who were sedentary or engaging in vigorous levels of physical activity. However, studies examining naturalistic infections could not control for exposure to antigens and actual URTI infection was not clinically confirmed. As the vaccination model allows a more controlled approach to test the effect of moderate physical activity on immune function, studies have investigated whether physical activity status is related to antibody response following vaccination as a more specific measure of immune function.

Initial cross sectional studies have found a positive relationship between cardiovascular fitness and vaccination response for influenza (Keylock et al, 2007) and novel antigen KLH (Smith et al, 2004). Unlike an influenza vaccination, which could elicit a secondary response due to exposure and previous vaccine history, a vaccination against KLH elicits purely a primary response within the immune system. Therefore, these initial results suggest that aerobic fitness is associated with both primary and secondary antibody responses in humans. However, these studies may be confounded by lifestyle factors, current health, or genetic

predisposition, which could also affect the relationship between exercise and vaccination response.

To further investigate the relationship between exercise and vaccination response, randomised, controlled exercise intervention studies have also been carried out. These studies can help elucidate causation between physical activity and antibody response, as physical activity behaviour is being manipulated and results can be compared to a control group. Kohut et al (2004b) found that a 10 month supervised physical activity intervention (3 times per week for 30 minutes at 65-75% of their heart rate reserve) in elderly participants improved antibody responses for two of the three influenza strains and cell mediated IFN- $\gamma$  response at 4 weeks post vaccination. The authors also found that this increase in IFN- $\gamma$  response, but not antibody response, was partly mediated by improvements in depression and sense of coherence (Kohut et al, 2005). This suggests that a physical activity intervention can improve vaccination response, and this may be partly due to changes in psychosocial health.

Grant et al (2008) found that the anti-KLH IgG1 and IgM antibody response at 2, 3 and 6 weeks post vaccination were increased in those who either exercised 3 times per week for 10 months, compared to a flexibility intervention, which served as an attention control group. However, these initial intervention studies had relatively low participant numbers (n= ~14). A subsequent larger scale study (n= 144) found that a 10 month supervised aerobic exercise intervention, using a similar protocol, increased seroprotection 24 weeks post vaccination, but not at 3 or 6 weeks post vaccination (Woods et al, 2009). This implies that a chronic exercise intervention may affect the maintenance of antibody response in an elderly population more so than the initial generation of the response.

Whilst these studies give convincing evidence that an aerobic exercise intervention can improve antibody response to both influenza and novel KLH antigen in the elderly, there are some disadvantages with this type of intervention. Due to the highly supervised and structured nature of this training protocol, it may be difficult and costly to implement within a clinical setting, whereas a less supervised physical activity intervention may be more suitable to administer to a wider target population. For example, 12 week physical activity consultation and pedometer based interventions have reported increases in physical activity behaviour (Kirk et al, 2007; Baker et al, 2008) and one review noted that similar increases in physical activity were found in both supervised and non-supervised exercise interventions (Dunn et al, 1999). Therefore, a less supervised intervention could increase physical activity and, consequentially, immune function in a similar way to previous supervised interventions. Furthermore, most previous studies have used a T cell dependent vaccination. Investigating the effect of a chronic training intervention upon a T cell independent response may help elucidate which pathways of the immune system may be improved following a physical activity intervention.

## **PSYCHOSOCIAL FACTORS AND ANTIBODY RESPONSE TO VACCINATION**

### **Animal literature**

Chronic stress, unlike acute stress which has generally shown a positive relationship with antibody response to vaccination, tends to show a negative relationship with antibody response. For example, Dhabhar and McEwen (1997) used a chronic (3-5 week) restraint stress and shaking protocol, prior to challenge with DFNB. Chronic stress was associated with a suppressed DTH response, compared to a non-stressed group. Silberman et al (2003) found that mice which underwent a 6 week chronic stress intervention had lower antibody levels to SRBCs, but no difference between conditions was seen for the T independent lipopolysaccharide response. As the evidence from chronic stress in animals suggests that prolonged stress is related to a reduced antibody response, some researchers have investigated this within the human population.

### **Human literature**

Initially, cross sectional studies investigated the relationship between psychosocial factors and antibody response to vaccination. For the most part, an inverse relationship between negative psychological factors, such as perceived stress, stressful life events and bereavement, and antibody response to both T cell dependent (Burns et al, 2002; 2003a; Phillips et al, 2005; 2006) and independent (Burns et al, 2003b; Phillips et al, 2005) vaccinations has been found in young, healthy adults. Studies have also shown that higher dispositional positive affect, larger social support networks, and higher marital satisfaction are related to better antibody responses to a variety of vaccinations (Marsland et al, 2003; Gallagher et al, 2008; Pressman et al, 2005; Phillips et al, 2006). Although there are a small number of studies which did not

find a relationship between psychological factors and antibody response (Marsland et al, 2001; Glaser et al, 1992), there is relatively consistent evidence that a negative correlation exists between psychosocial factors and antibody response.

The caregiver-control model allows a comparison between caregivers, who experience prolonged chronic stress, with non-stressed age and sex-matched controls. (Kiecolt-Glaser et al, 1987; 1996; Gallagher et al, 2009a; 2009b, Vedhara et al, 1999; Li et al, 2007; Glaser et al, 2000). For example, Kiecolt-Glaser et al (1996) found that elderly caregivers had a poorer antibody response 4 weeks following influenza vaccination, compared to age matched controls. Furthermore, Glaser et al (2000) found a negative association between antibody response to pneumococcal vaccination, which stimulates a T cell independent immune response, and caregiving. However, younger chronically stressed populations, such as parental caregivers of children with disabilities, also mount reduced responses to both influenza and pneumonia vaccinations, compared to parents of typically developing children (Gallagher et al, 2009a; 2009b). This evidence implies that chronic stress has a detrimental effect upon the antibody response following vaccination for both T dependent and independent vaccinations. Despite this, the mechanisms behind this immune suppression are less clear and not widely investigated. Vedhara et al (1999) found that carers had a significantly higher mean area under the curve for cortisol values at baseline, 3 and 6 months. Furthermore, an inverse relationship between salivary cortisol and antibody response to one of the influenza vaccination strains was found at 2 weeks post vaccination. This would suggest that chronic activation of the hypothalamic pituitary axis (HPA) axis during prolonged caregiving may act as a mediator of reduced antibody response. Caregivers have shown higher levels of serum IL-6, compared to controls (Segerstrom et al, 2008), but this was not associated with antibody response (Segerstrom et al, 2008; Kiecolt-Glaser et al, 1996). In

conclusion, most studies suggest that negative psychological factors and chronic stressful situations, such as caregiving, reduce antibody response to both T dependent and independent vaccinations. However, another population which tend to experience high levels of stress are HIV+ patients (Bing et al, 2001; Leserman, 2008). In addition, due to the nature of HIV infection, there is some evidence that patients tend to already have a poorer antibody response to vaccination, depending on the vaccine type and the patients' CD4+ count (Rivas et al, 2007). However, it is not clear whether suffering from higher psychological distress would further decrease antibody response to vaccination in HIV+ patients. Therefore, research investigating whether psychosocial factors, such as depression and perceived stress, or health behaviours, such as physical activity levels, affect antibody response to vaccination in an HIV+ population is warranted.



## **PSYCHOSOCIAL FACTORS, PHYSICAL ACTIVITY AND IMMUNE FUNCTION IN AN HIV+ POPULATION**

In 2009, an estimated 86,500 people were living in the UK with the human immunodeficiency virus (HIV) and there were 6630 newly diagnosed HIV infections (Presanis et al, 2010). HIV is one of a subgroup of slow acting retroviruses called lentiviruses (Chiu et al, 1985). There are two types of HIV specific retrovirus: HIV-1 and HIV-2. HIV-1 is more common and results in acquired immune deficiency syndrome (AIDS) in more patients (de Silva et al, 2008), whereas HIV-2 is endemic in West Africa and has prolonged periods of asymptomatic infection (HPA, CDSC, 2003; Poulsen et al, 1997). Once inside the body, the HIV molecule attaches to the CD4+ antigen and co-receptors to form a complex which transfers through to the cytoplasm, where the HIV ribonucleic acid (RNA) is converted to deoxyribonucleic acid (DNA). This DNA moves to the cell nucleus to replicate and form viral proteins via transcription and translation. The virus then pinches off the CD4+ cells' outer membrane and 'buds' out of the cell; this HIV-1 RNA in plasma is known as viral load (Smith et al, 2006; Mellors et al, 1995; 1997). Following acute activation, where an initial sharp fall occurs, viral load then rests at a 'set point', which positively predicts the speed of a patient's progression to AIDS; the higher a patient's set point, the faster the progression to AIDS (Mellors et al, 1995).

HIV causes a decline in CD4+ T cell numbers, which are important in activating and maintaining an immune response. The initial rapid depletion of CD4+ cells begins in the gut and lymphoid organs, where CD4+ depletion is higher than in peripheral blood, before moving into a chronic stage of infection where the persistent direct activation of these cells reduces their capacity to regenerate over time (Forsman and Weiss, 2008; Grossman et al, 2006). CD4+ cells in peripheral blood act as a marker of disease progression; once CD4+

cells fall below 200 cells/ $\mu$ l the immune system is heavily compromised and opportunistic infections can occur, leading to AIDS (Klimas et al, 2008; Ho et al, 1995).

AIDS defining illnesses include opportunistic diseases such as tuberculosis, pneumo-cystitis, cytomegalovirus, cerebral toxoplasma and Candida. Not only can AIDS result in opportunistic infection, but opportunistic infections can also accelerate progression to AIDS. For example HIV progression may cause cytomegalovirus (CMV) reactivation, which in turn can promote HIV pathogenicity (Griffiths, 2006). In addition to these pathways, there is also some evidence that lifestyle factors, such as psychological health and physical activity status, may also affect HIV+ progression and AIDS related morbidity.

### **Psychosocial factors and HIV progression**

Depression is one of the most commonly reported mental disorders among HIV patients (Whetten et al, 2008) and, as a result, has been one of the most examined psychosocial variables in relation to disease status. Schroeksnadel et al (2008) found that patients without depression, had significantly higher CD4+ counts and a lower plasma neopterin concentration, which is a marker of immune activation, compared to those who were depressed. Kaharuza et al (2006) also found that more depressive symptoms were significantly related to a lower CD4+ count among Ugandan HIV infected patients, regardless of age, education, gender or source of income. Their findings suggested that this relationship became more pronounced when the CD4+ count falls below 100 cells/ $\mu$ l. Although most literature suggests that higher levels of depression have a negative relationship with HIV disease status (Leserman et al, 2008), the direction of the causation is not clear; high levels of depression may predict a lower CD4+ count at follow up, or depression may occur as a result

of having lower CD4+ counts. However, longitudinal evidence shows that higher levels of depression significantly predict subsequent higher viral load, faster CD4+ cell decline and progression to AIDS (Ickovics et al, 2001; Evans et al, 1997; Farinpour et al, 2003; Ironson et al 2005; Leserman et al, 1999; Leserman et al, 2003; Leserman et al, 2007). The largest longitudinal study followed up 765 women every 6 months over a period of 7 years and found that chronically depressed patients who had <200 $\mu$ l CD4+ cell count at baseline were twice as likely to die during the 7 year follow up period, compared to patients with limited or no depressive symptoms (Ickovics et al, 2001). Although some studies have failed to find a significant relationship between depression and factors related to HIV status and mortality, these have tended to be when using non-standard depression cut offs, which may have falsely categorised HIV+ patients as depressed (Lyketsos et al, 1993), and short follow up periods (Eich-Hochli et al, 1997; Perry et al, 1992; Lyketsos et al, 1993; Vedhara et al, 1999).

Quality of life, trauma, stressful life events and perceived stress have also been given some attention as predictors of HIV disease status and have found significant relationships for both cross sectional (Schroeksnadel et al, 2008) and longitudinal research (Ironson et al, 2005; Golub et al, 2003; Evans et al, 1997; Kemeney et al, 1995; Leserman et al 2002; Leserman et al, 2007) in a variety of HIV+ populations. For example, Schroeksnadel et al (2008) found that a lower quality of life score was associated with a higher viral load, but not CD4+ count, in 152 HIV+ patients and Golub et al (2003) found that, in a cohort of injection drug users, higher levels of distress resulted in a faster time to AIDS diagnosis. Greeson and colleagues (2008) found that higher levels of psychological distress was significantly related to a higher viral load and lower CD4+ counts, accounting for 67% of the variation in HIV disease severity. This model did not support a reverse directionality hypothesis, indicating that greater distress was more likely to predict greater disease severity than *vice versa*. This

evidence suggests that both measures of HIV progression, such as CD4+ count and viral load, and conditions associated with HIV infection, such as time to AIDS diagnosis, are related to quality of life and measures of psychosocial distress.

Social support has also been investigated in relation to HIV disease progression and health status, which has yielded mixed results. Leserman et al (2002) found that higher cumulative social support was associated with slower progression to AIDS or AIDS clinical condition over 9 years, whereas six studies have reported no significant social support predictors of HIV health outcomes (Ironson and Hayward, 2008). However, this may be due to the cumulative nature of the social support score and long follow up period in Leserman's (2002) study. Furthermore, some studies have only shown a protective effect of social support when disease progression is advanced (Patterson et al, 1996; Solano et al, 1993), suggesting social support is only important once patients are particularly unwell. Therefore, although results are mixed, social support appears to have some beneficial effects upon HIV disease progression. These results suggest that depression, stress, quality of life and social support predict HIV progression, long term health outcomes and mortality. As both psychosocial factors and physical activity levels affect measures of immune function, such as antibody response to vaccination, within the healthy population, it may also be meaningful to examine the effects of physical activity within HIV+ patients.

## **Exercise behaviour and HIV progression**

### **Cross sectional evidence**

Other factors, such as physical activity status, may also affect HIV progression. For example, physical activity can help HIV patients to manage their illness by slowing muscle wasting, improving cardiovascular and lipid abnormalities and addressing issues with life situations, such as inability to work (Macallan, 1999; Terry et al, 1999; O'Brien et al, 2010). Physical activity may also help improve psychological health and cope with illness (LaPerriere et al, 1990; 1991, Tkachuk and Martin, 1999; Neidig et al, 2003; Gauvin et al, 1996; Morgan and Goldston, 1997). Clearly, physical activity has benefits for the HIV+ population and is important to address as fewer HIV+ patients achieve the physical activity guidelines of 30 minutes of moderate physical activity, 3 times per week, compared to the general population (Clingerman et al, 2003; Filipas et al, 2008). If physical activity improves some illness-related physical functioning aspects of HIV+ infection, such as pain and fatigue, and also patients' psychological status, it is possible that it may also be associated with HIV disease progression.

Although one early study found that higher physical activity levels were significantly associated with a higher CD4+ count and lower viral load (Pothoff et al, 1994), more recent studies have found mixed results. Bopp et al (2004) measured objective physical activity in 66 HIV patients using a mini motion logger wrist actigraph for 3 days and found an inverse relationship between physical activity and viral load, but not CD4+ count. However, most cross sectional studies have failed to find an association between CD4+ count or viral load and physical activity status (Ramírez-Marerro et al, 2004; Smit et al, 2006). Oursler et al (2006) measured the aerobic capacity of 32 HIV+ older adults, compared to a healthy age-

matched control group and found that VO<sub>2</sub> peak did not correlate with CD4+ count or viral load in either group. This may be because the majority of participants were on anti-retroviral therapy (ART), which slows CD4+ T cell depletion, and therefore had relatively high CD4+ counts. As one pre-ART study found a relationship between CD4+ count and viral load (Pothoff et al, 1994), it may be that physical activity only affects HIV status once CD4+ counts are below a certain level. Furthermore, studies have tended to have relatively small participant numbers, which may contribute to the mostly negative results. It is possible that intervention studies, where HIV+ patients' physical activity levels are increased, may show a stronger association with HIV progression.

### **Intervention studies**

LaPerriere et al (1991) carried out a supervised exercise intervention (45 minute interval training session 3 times per week for 10 weeks) in a risk group of gay men who were yet to know their HIV serostatus; this result was given at week 6 of the intervention. Results showed that the intervention group increased their aerobic capacity and, the week following serostatus notification, the HIV positive participants had higher CD4+ counts compared to the seropositive control participants. Perna et al (1999) found that a 12 week aerobic exercise intervention of 45 minute interval training sessions 3 times per week improved cardiovascular fitness and CD4+ counts, compared to the control group. Interestingly, participants who did not comply with the intervention had a faster decline in CD4+ counts compared both to those who did adhere and to the control group. It is possible this lack of adherence prevented the intervention participants from adapting to the physical strain of the exercise, thus explaining the adverse effect on the CD4+ count. In contrast, some exercise intervention studies have

found no significant change in any immune parameters relating to HIV status (Rigsby et al, 1992; Smith et al, 2001; Bagis et al, 2002). It is possible that these mixed results could have been due to small sample sizes, a relatively low aerobic exercise prescription (only 20 minutes of cycling exercise at 60-80% heart rate reserve 3 days per week) or the different baseline fitness of participants. Furthermore, the relatively high attrition rates and non compliance in these studies may have prevented a significant change in immunological variables. One plausible mechanism by which aerobic exercise interventions could influence immune function, is by changing cardiovascular fitness and body composition (O'Brien et al, 2010; Moir and Fauci, 2009), but further studies are needed to determine whether this would affect HIV progression. The literature gives mixed evidence regarding the effect of physical activity on immunological variables relating to HIV progression. However, it is not clear how other measures of immune function, such as antibody response to vaccination, may be affected by psychosocial factors and physical activity status in HIV+ patients, and future research in this area is warranted.

### **Vaccination responses in HIV+ patients**

Vaccinations may be used to help HIV+ patients, who have an increased susceptibility to and higher mortality rates from various infectious diseases (Rivas et al. 2007), gain some protection from a number of life threatening diseases. HIV+ patients generally tend to experience poorer antibody responses to various vaccinations, compared to the healthy population (Rodriguez-Barradas et al, 1996; Opravil et al, 1991; Rivas et al, 2007). Although this may be explained by the immunological changes associated with HIV infections, such as B cell dysfunction and T cell depletion (Kroon et al, 1994; Moir and Fauci, 2009),

psychological factors and physical activity levels may also affect antibody response as in the healthy population. As the effects of both stress and exercise upon antibody response have been shown in immunosuppressed populations, such as the elderly, it is possible that an immunosuppressed HIV+ population may also show relationships between antibody responses and psychosocial and physical activity levels. Therefore, this warrants investigation into whether factors such as psychosocial stress and physical activity can affect antibody response to vaccination in HIV+ patients.



## **OVERVIEW OF THESIS**

This thesis will incorporate the vaccination model in three studies which investigate the effects of stress and exercise upon antibody response in healthy young, older, and HIV+ participants. The first two studies are large intervention trials which were carried out at the School of Sport and Exercise Sciences and the third is an observational study in a clinical population. Chapter Two examined whether moderate aerobic exercise can be used as an adjuvant to vaccination in young (18-30yrs) and older (50-64yrs) healthy adults. A brisk walk was employed as the mode of exercise prior to pneumococcal and half-dose influenza vaccination, and antibody responses at 4 weeks post vaccination were examined. Chapter Three investigated whether a moderate physical activity training intervention improved antibody response to vaccination, as a measure of general immune function, in sedentary middle-aged women. Physiological and psychological measures were also investigated pre and post intervention to examine any possible mediating factors between lifestyle physical activity and antibody response. Finally, Chapter Four is a sub-study of a trial conducted by University Hospitals Birmingham which investigated antibody response to a number of vaccinations in HIV+ patients, compared to a non-HIV cohort. Our study evaluated whether health behaviours, psychosocial factors and physical activity levels affect the extent of antibody response to vaccination in HIV+ patients. It was hypothesised that:

- 1) an acute moderate physical activity intervention would improve antibody response to an influenza and pneumococcal vaccination, compared to a control group, and this improvement would be greater in an older adult population, compared to a younger cohort;

- 2) a chronic moderate physical activity intervention would increase physical activity levels and result in an improvement in antibody response to a pneumococcal vaccination, compared to an educational control group;
- 3) psychosocial factors, such as high depression and life event stress, and low physical activity levels, would be associated with a lower antibody response to vaccination in HIV+ patients.

## REFERENCES

- Abbas, A. K., Lichtman, A. H., 2006. *Basic Immunology; Functions and Disorders of the Immune System*, Elsevier, Philadelphia. 2nd edition.
- Ader, R., 1981. *Psychoneuroimmunology*, Academic Press, New York.
- Baigis, J., Korniewicz, D.M., Chase, G., Butz, A., Jacobson, D., Wu, A.W., 2002. Effectiveness of a home-based exercise intervention for HIV-infected adults: A randomized trial. *J Assoc Nurses AIDS Care* 13, 33-45.
- Baker, G., Gray, S., Wright, A., Fitzsimons, C., Nimmo, M., Lowry, R., Mutrie, N., the Scottish Physical Activity Research Collaboration (SPARColl), 2008. The effect of a pedometer-based community walking intervention "Walking for Wellbeing in the West" on physical activity levels and health outcomes: a 12-week randomized controlled trial. *Int J Behav Nutr Phys Act* 5, 44.
- Barnes, C.A., Forster, M.J., Fleshner, M., Ahanotu, E.N., Laudenslager, M.L., Mazzeo, R.S., Maier, S.F., Lal, H., 2001. Exercise does not modify spatial memory, brain autoimmunity, or antibody response in aged F-344 rats. *Neurobiol Aging* 12, 47-53.
- Bing, E.G., Burnam, M.A., Longshore, D., Fleishman, J.A., Sherbourne, C.D., London, A.S., Turner, B.J., Eggan, F., Beckman, R., Vitiello, B., Morton, S.C., Orlando, M., Bozette, S.A., Ortiz-Barron, L., Shapiro, M., 2001. Psychiatric disorders and drug use among human immunodeficiency virus-infected adults in the United States. *Arch Gen Psychiatry* 58, 721-728.
- Bopp, C.M., Phillips, K.D., Fulk, L.J., Dudgeon, W.D., Sowell, R., Hand, G.A., 2004. Physical activity and immunity in HIV-infected individuals. *AIDS Care* 16, 387-393.
- Brunswick, M., Finkelman, F.D., Hight, P.F., Inman, J.K., Dintzis, H.M., Mond, J.J., 1988. Picogram quantities of anti-Ig antibodies coupled to dextran induce B cell proliferation. *J Immunol* 140, 3364-3372.
- Brunsgaard, H., Hartkopp, A., Mohr, T., Konradsen, H., Heron, I., Mordhorst, C.H., Pedersen, B.K., 1997. In vivo cell-mediated immunity and vaccination response following prolonged, intense exercise. *Med Sci Sports Exerc* 29, 1176-81.
- Burns, V.E., Carroll, D., Ring, C., Harrison, L.K., Drayson, M., 2002. Stress, coping, and hepatitis B antibody status. *Psychosom Med* 64, 287-293.
- Burns, V.E., Carroll, D., Ring, C., Drayson, M., 2003a. Antibody response to vaccination and psychosocial stress in humans: relationships and mechanisms. *Vaccine* 21, 2523-2534.
- Burns, V.E., Carroll, D., Drayson, M., Whitham, M., Ring, C., 2003b. Life events, perceived stress and antibody response to influenza vaccination in young, healthy adults. *J Psychosom Res* 55, 569-572.

- Burns, V.E., Gallagher, S., 2010. Antibody response to vaccination as a marker of in vivo immune function in psychophysiological research. *Neurosci Biobehav Rev* 35, 122-126.
- Campbell, J.P., Edwards, K.M., Ring, C., Drayson, M.T., Bosch, J.A., Inskip, A., Long, J.E., Pulsford, D., Burns, V.E., 2010. The effects of vaccine timing on the efficacy of an acute eccentric exercise intervention on the immune response to an influenza vaccine in young adults. *Brain Behav Immun* 24, 236-242.
- Chiu, I.M., Yaniv, A., Dahlberg, J.E., Gazit, A., Skuntz, S.F., Tronick, S.R., Aaronson, S.A., 1985. Nucleotide sequence evidence for relationship of AIDS retrovirus to lentiviruses. *Nature* 317, 366-368.
- Clingerman, E.M., 2003. Participation in physical activity by persons living with HIV disease. *J Assoc Nurses AIDS Care* 14, 59-70.
- Coleman, K.J., Rager, D.R., 1993. Effects of voluntary exercise on immune function in rats. *Physiol Behav* 54, 771-774.
- de Silva, T.I., Cotten, M., Rowland-Jones, S.L., 2008. HIV-2: the forgotten AIDS virus. *Trends Microbiol* 16, 588-595.
- Dhabhar, F.S., McEwen, B.S., 1996. Stress-induced enhancement of antigen-specific cell-mediated immunity. *J Immunol* 156, 2608-2615.
- Dhabhar, F.S., McEwen, B.S., 1997. Acute stress enhances while chronic stress suppresses cell-mediated immunity in vivo: A potential role for leukocyte trafficking. *Brain Behav Immun* 11, 286-306.
- Dhabhar, F.S., McEwen, B.S., 1999. Enhancing versus suppressive effects of stress hormones on skin immune function. *Proc Natl Acad Sci U S A* 96, 1059-1064.
- Dunn, A.L., Marcus, B.H., Kampert, J.B., Garcia, M.E., Kohl III, H.W., Blair, S.N., 1999. Comparison of lifestyle and structured interventions to increase physical activity and cardiorespiratory fitness: A randomized trial. *JAMA* 281, 327-334.
- 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 Behav Immun* 20, 159-168.
- Edwards, K.M., Burns, V.E., Ring, C., Carroll, D., 2006. Individual differences in the interleukin-6 response to maximal and submaximal exercise tasks. *J Sports Sci* 24, 855-862.
- Edwards, K.M., Burns, V.E., Allen, L.M., McPhee, J.S., Bosch, J.A., Carroll, D., Drayson, M., Ring, C., 2007. Eccentric exercise as an adjuvant to influenza vaccination in humans. *Brain Behav Immun* 21, 209-217.

- Edwards, K.M., Burns, V.E., Adkins, A.E., Carroll, D., Drayson, M., Ring, C., 2008. Meningococcal A vaccination response is enhanced by acute stress in men. *Psychosom Med* 70, 147-151.
- Edwards, K.M., Campbell, J.P., Ring, C., Drayson, M.T., Bosch, J.A., Downes, C., Long, J.E., Lumb, J.A., Merry, A., Paine, N.J., Burns, V.E., 2010. Exercise intensity does not influence the efficacy of eccentric exercise as a behavioural adjuvant to vaccination. *Brain Behav Immun* 24, 623-630.
- Edwards, K.M., Campbell, J.P., 2011. Acute exercise as an adjuvant to influenza vaccination. *Am J Lifestyle Med* 5, 512-517.
- Eich-Höchli, D., Niklowitz, M.W., Lüthy, R., Opravil, M., 1997. Are immunological markers, social and personal resources, or a complaint-free state predictors of progression among HIV-infected patients? *Acta Psychiatr Scand* 95, 476-484.
- Eskola, J., Ruuskanen, O., Soppi, E., Viljanen, M.K., Jarvinen, M., Toivonen, H., Kouvalainen, K., 1978. Effect of sport stress on lymphocyte transformation and antibody formation. *Clin Exp Immunol* 32, 339-345.
- Evans, D.L., Leserman, J., Perkins, D.O., Stern, R.A., Murphy, C., Zheng, B., Gettes, D., Longmate, J.A., Silva, S.G., van der Horst, C.M., Hall, C.D., Folds, J.D., Golden, R.N., Petitto, J.M., 1997. Severe life stress as a predictor of early disease progression in HIV infection. *Am J Psychiatry* 154, 630-634.
- Farinpour, R., Miller, E.N., Satz, P., Selnes, O.A., Cohen, B.A., Becker, J.T., Skolasky, R.L., Visscher, B.R., 2003. Psychosocial risk factors of HIV morbidity and mortality: Findings from the Multicenter AIDS Cohort Study (MACS). *J Clin Exp Neuropsychol* 25, 654-670.
- Fillipas, S., Bowtell-Harris, C.A., Oldmeadow, L.B., Cicuttini, F., Holland, A.E., Cherry, C.L., 2008. Physical activity uptake in patients with HIV: who does how much? *Int J STD AIDS* 19, 514-518.
- Fleshner, M., Hermann, J., Lockwood, L.L., Laudenslager, M.L., Watkins, L.R., Maier, S.F., 1995. Stressed rats fail to expand the CD45RC+CD4+ (Th1-Like) T cell subset in response to KLH: Possible involvement of IFN-gamma. *Brain Behav Immun* 9, 101-112.
- Fleshner, M., Nguyen, K.T., Cotter, C.S., Watkins, L.R., Maier, S.F., 1998. Acute stressor exposure both suppresses acquired immunity and potentiates innate immunity. *Am J Physiol Regul Integr Comp Physiol* 275, R870-R878.
- Forsman, A., Weiss, R.A., 2008. Why is HIV a pathogen? *Trends Microbiol* 16, 555-560.
- Gallagher, S., Phillips, A.C., Ferraro, A.J., Drayson, M.T., Carroll, D., 2008. Psychosocial factors are associated with the antibody response to both thymus-dependent and thymus-independent vaccines. *Brain Behav Immun* 22, 456-460.

- Gallagher, S., Phillips, A.C., Drayson, M.T., Carroll, D., 2009. Caregiving for children with developmental disabilities is associated with a poor antibody response to influenza vaccination. *Psychosom Med* 71, 341-344.
- Gallagher, S., Phillips, A.C., Drayson, M.T., Carroll, D., 2009. Parental caregivers of children with developmental disabilities mount a poor antibody response to pneumococcal vaccination. *Brain Behav Immun* 23, 338-346.
- Gallucci, S., Lolkema, M., Matzinger, P., 1999. Natural adjuvants: Endogenous activators of dendritic cells. *Nat Med* 5, 1249-1255.
- Gallucci, S., Matzinger, P., 2001. Danger signals: SOS to the immune system. *Curr Opin Immunol* 13, 114-119.
- Gauvin, L., Rejeski, W.J., Norris, J.L., 1996. A naturalistic study of the impact of acute physical activity on feeling states and affect in women. *Health Psychol* 15, 391-397.
- Gazda, L.S., Smith, T., Watkins, L.R., Maier, S.F., Fleshner, M., 2003. Stressor exposure produces long-term reductions in antigen-specific T and B cell responses. *Stress* 6, 259-267.
- 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. *Psychosom Med* 54, 22-29.
- Glaser, R., Sheridan, J., Malarkey, W.B., MacCallum, R.C., Kiecolt-Glaser, J.K., 2000. Chronic stress modulates the immune response to a pneumococcal pneumonia vaccine. *Psychosom Med* 62, 804-807.
- Gleeson, M., 2006. *Immune Function in Sport and Exercise*, Edinburgh, Elsevier.
- Golub, E.T., Astemborski, J.A., Hoover, D.R., Anthony, J.C., Vlahov, D., Strathdee, S.A., 2003. Psychological distress and progression to AIDS in a cohort of injection drug users. *J Acquir Immune Defic Syndr* 32, 429-434.
- 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 Behav Immun* 22, 923-932.
- Greeson, J.M., Hurwitz, B.E., Llabre, M.M., Schneiderman, N., Penedo, F.J., Klimas, N.G., 2008. Psychological distress, killer lymphocytes and disease severity in HIV/AIDS. *Brain Behav Immun* 22, 901-911.
- Grossman, Z., Meier-Schellersheim, M., Paul, W.E., Picker, L.J., 2006. Pathogenesis of HIV infection: what the virus spares is as important as what it destroys. *Nat Med* 12, 289-295.

- Grubeck-Loebenstien, B., Della Bella, S., Iorio, A.M., Michel, J.P., Pawelec, G., Solana, R., 2009 Immunosenescence and vaccine failure in the elderly. *Aging Clin Exp Res* 21, 201-209
- Hirose, L., Nosaka, K., Newton, M., Laveder, A., Kano, M., Peake, J., Suzuki, K., 2004. Changes in inflammatory mediators following eccentric exercise of the elbow flexors. *Exerc Immunol Rev* 10, 75-90.
- Ho, D.D., Neumann, A.U., Perelson, A.S., Chen, W., Leonard, J.M., Markowitz, M., 1995. Rapid turnover of plasma virions and CD4 lymphocytes in HIV-1 infection. *Nature* 373, 123-126.
- Health Protection Agency, Communicable Disease Surveillance Centre, 2003.
- Ickovics, J.R., Hamburger, M.E., Vlahov, D., Schoenbaum, E.E., Schuman, P., Boland, R.J., Moore, J., for the HIV Epidemiology Research Study Group, 2001. Mortality, CD4 Cell count decline, and depressive symptoms among HIV-seropositive women: Longitudinal analysis from the HIV epidemiology research study. *JAMA* 285, 1466-1474.
- Ironson, G., O'Cleirigh, C., Fletcher, M.A., Laurenceau, J.P., Balbin, E., Klimas, N., Schneiderman, N., Solomon, G., 2005. Psychosocial factors predict CD4 and viral load change in men and women with human immunodeficiency virus in the era of highly active antiretroviral treatment. *Psychosom Med* 67, 1013-1021.
- Ironson, G., Hayward, H.S., 2008. Do positive psychosocial factors predict disease progression in HIV-1? A review of the evidence. *Psychosom Med* 70, 546-554.
- Kaharuza, F., Bunnell, R., Moss, S., Purcell, D., Bikaako-Kajura, W., Wamai, N., Downing, R., Solberg, P., Coutinho, A., Mermin, J., 2006. Depression and CD4 cell count among persons with HIV infection in Uganda. *AIDS Behav* 10, 105-111.
- Kapasi, Z.F., Catlin, P.A., Joyner, D.R., Lewis, M.L., Schwartz, A.L., Townsend, E.L., 2000. The effects of intense physical exercise on secondary antibody response in young and old mice. *Phys Ther* 80, 1076-1086.
- Kapasi, Z.F., Catlin, P.A., Beck, J., Roehling, T., Smith, K., 2001. The role of endogenous opioids in moderate exercise training-induced enhancement of the secondary antibody response in mice. *Phys Ther* 81, 1801-1809.
- Karp, J.D., Smith, J., Hawk, K., 2008. Restraint stress augments antibody production in cyclophosphamide-treated mice. *Physiol Behav* 70, 271-278.
- Kaufman, J.C., Harris, T.J., Higgins, J., Maisel, A.S., 1994. Exercise-induced enhancement of immune function in the rat. *Circulation* 90, 525-532.
- Kemeny M.E., Dean, L., 1995 Effects of AIDS-related bereavement on HIV progression among New York City gay men. *AIDS Educ Prev* 7, 36-47.

- Keylock, K.T., Lowder, T., Leifheit, K.A., Cook, M., Mariani, R.A., Ross, K., Kim, K., Chapman-Novakofski, K., McAuley, E., Woods, J.A., 2007. Higher antibody, but not cell-mediated, responses to vaccination in high physically fit elderly. *J Appl Physiol* 102, 1090-1098.
- Kiecolt-Glaser, J.K., Glaser, R., Shuttleworth, E.C., Dyer, C.S., Ogrocki, P., Speicher, C.E., 1987. Chronic stress and immunity in family caregivers of Alzheimer's disease victims. *Psychosom Med* 49, 523-535.
- 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. *Proc Natl Acad Sci U S A* 93, 3043-3047.
- Kirk, A., Barnett, J., Mutrie, N., 2007. Physical activity consultation for people with Type 2 diabetes. Evidence and guidelines. *Diabetic Med* 24, 809-816.
- Klimas, N., Koneru, A.O., Fletcher, M.A., 2008. Overview of HIV. *Psychosom Med* 70, 523-530.
- Kohut, M.L., Lee, W., Martin, A., Arnston, B., Russell, D.W., Ekkekakis, P., Yoon, K.J., Bishop, A., Cunnick, J.E., 2005. The exercise-induced enhancement of influenza immunity is mediated in part by improvements in psychosocial factors in older adults. *Brain Behav Immun* 19, 357-366.
- Kohut, M.L., Arntson, B.A., Lee, W., Rozeboom, K., Yoon, K.J., Cunnick, J.E., McElhaney, J., 2004a. Moderate exercise improves antibody response to influenza immunization in older adults. *Vaccine* 22, 2298-2306.
- Kohut, M.L., Thompson, J.R., Lee, W., Cunnick, J.E., 2004b. Exercise training-induced adaptations of immune response are mediated by beta-adrenergic receptors in aged but not young mice. *J Appl Physiol* 96, 1312-1322.
- Krakauer, T., 1995. Levels of interleukin 6 and tumor necrosis factor in serum from humans vaccinated with live, attenuated *Francisella tularensis*. *Clin. Diagn. Lab. Immunol.* 2, 487-488.
- Kroon, F.P., van Dissel, J.T., de Jong, J.C., van Furth, R., 1994. Antibody response to influenza, tetanus and pneumococcal vaccines in HIV-seropositive individuals in relation to the number of CD4+ lymphocytes. *AIDS* 8, 469-476.
- LaPerriere, A., Fletcher, M.A., Antoni, M.H., Klimas, A., Ironson, G., Schneiderman, N., 1991. Aerobic exercise training in an AIDS risk group. *Int J Sports Med* 12, S53-S57.
- LaPerriere, A.R., Antoni, M.H., Schneiderman, N., Ironson, G., Klimas, N., Caralis, P., Fletcher, M.A., 1990. Exercise intervention attenuates emotional distress and natural killer cell decrements following notification of positive serologic status for HIV-1. *Appl Psychophysiol Biofeedback* 15, 229-242.



- Laudenslager, M.L., Fleshner, M., Hofstadter, P., Held, P.E., Simons, L., Maier, S.F., 1988. Suppression of specific antibody production by inescapable shock: stability under varying conditions. *Brain Behav Immun* 2, 92-101.
- Leserman, J., Jackson, E.D., Petitto, J.M., Golden, R.N., Silva, S.G., Perkins, D.O., Cai, J., Folds, J.D., Evans, D.L., 1999. Progression to AIDS: The effects of stress, depressive symptoms, and social support. *Psychosom Med* 61, 397-406.
- Leserman, J., Petitto, J.M., Gu, H., Gaynes, B.N., Barroso, J., Golden, R.N., Perkins, D.O., Folds, J.D., Evans, D.L., 2002. Progression to AIDS, a clinical AIDS condition and mortality: psychosocial and physiological predictors. *Psychol Med* 32, 1059-1073.
- Leserman, J., 2003. HIV disease progression: depression, stress, and possible mechanisms. *Biol Psychiatry* 54, 295-306.
- Leserman, J., Pence, B.W., Whetten, K., Mugavero, M.J., Thielman, N.M., Swartz, M.S., Stangl, D., 2007. Relation of lifetime trauma and depressive symptoms to mortality in HIV. *Am J Psychiatry* 164, 1707-1713.
- Leserman, J., 2008. Role of depression, stress, and trauma in HIV disease progression. *Psychosom Med* 70, 539-545.
- Li, J., Cowden, L.G., King, J.D., Briles, D.A., Schroeder, H.W., Jr., Stevens, A.B., Perry, R.T., Chen, Z., Simmons, M.S., Wiener, H.W., Tiwari, H.K., Harrell, L.E., Go, R.C.P., 2007. Effects of chronic stress and interleukin-10 gene polymorphisms on antibody response to tetanus vaccine in family caregivers of patients with Alzheimer's disease. *Psychosom Med* 69, 551-559.
- Liu, Y.G., Wang, S.Y., 1987. The enhancing effect of exercise on the production of antibody to *Salmonella typhi* in mice. *Immunol Lett* 14, 117-120.
- Lyketsos, C.G., Hoover, D.R., Guccione, M., Senterfitt, W., Dew, M.A., Wesch, J., VanRaden, M.J., Treisman, G.J., Morgenstern, H., the Multicenter AIDS Cohort Study (MACS), 1993. Depressive symptoms as predictors of medical outcomes in HIV infection. *JAMA* 270, 2563-2567.
- Macallan, D.C., 1999. Wasting in HIV Infection and AIDS. *J Nutr* 129, 238S-242S.
- 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 Psychol* 20, 4-11.
- Marsland, A.L., Cohen, S., Rabin, B.S., Manuck, S.B., 2006. Trait positive affect and antibody response to hepatitis B vaccination. *Brain Behav Immun* 20, 261-269.
- Mellors, J.W., Kingsley, L.A., Rinaldo, C.R., Todd, J.A., Hoo, B.S., Kokka, R.P., Gupta, P., 1995. Quantitation of HIV-1 RNA in plasma predicts outcome after seroconversion. *Ann Intern Med* 122, 573-579.

- Mellors, J.W., Munoz, A., Giorgi, J.V., Margolick, J.B., Tassoni, C.J., Gupta, P., Kingsley, L.A., Todd, J.A., Saah, A.J., Detels, R., Phair, J.P., Rinaldo, J., 1997. Plasma viral load and CD4+ lymphocytes as prognostic markers of HIV-1 infection. *Ann Intern Med* 126, 946-954.
- Meyer, R.J., Haggerty, R.J., 1962. Streptococcal infections in families. *Pediatrics* 29, 539-549.
- Millán, S., González-Quijano, M.I., Giordano, M., Soto, L., Martín, A.I., López-Calderón, A., 1996. Short and long restraint differentially affect humoral and cellular immune functions. *Life Sci* 59, 1431-1442.
- Moir, S., Fauci, A.S., 2009. B cells in HIV infection and disease. *Nat Rev Immunol* 9, 235-245.
- Mond, J.J., Sehgal, E., Sachs, D.H., Paul, W.E., 1979. Expression of Ia Antigen on Adult and Neonatal B Lymphocytes Responsive to Thymus-Independent Antigens. *J Immunol* 123, 1619-1623.
- Mond, J.J., Vos, Q., Lees, A., Snapper, C.M., 1995. T cell independent antigens. *Curr Opin Immunol* 7, 349-354.
- Morgan, W. P., Goldston, S. E., 1987. Exercise and mental health. Washington, DC, Hemisphere.
- Neidig, J.L., Smith, B.A., Brashers, D.E., 2003. Aerobic exercise training for depressive symptom management in adults living with HIV infection. *J Assoc Nurses AIDS Care* 14, 30-40.
- Nieman, D.C., 1994. Exercise, upper respiratory tract infection, and the immune system. *Med Sci Sports Exerc* 26, 128-139.
- O'Brien K., Nixon S., Tynan A.M., Glazier R.H., 2010. Aerobic exercise interventions for adults living with HIV/AIDS. *Cochrane Database Syst Rev* 4, 1-35.
- Opravil, M., Fierz, W., Matter, L., Blaser, J., Lüthy, R., 1991. Poor antibody response after tetanus and pneumococcal vaccination in immunocompromised, HIV-infected patients. *Clin Exp Immunol* 84, 185-189.
- Oursler, K.K., Sorkin, J.D., Smith, B.A., Katznel, L.I., 2006. Reduced aerobic capacity and physical functioning in older HIV-infected men. *AIDS Res Hum Retroviruses* 22, 1113-1121.
- Patterson, T.L., Shaw, W.S., Semple, S.J., Cherner, M., McCutchan, J.A., Hampton Atkinson, J., Grant, I., Nannis, E., and HIV Neurobehavioral Research Center (HNRC) Group., 1996. Relationship of psychosocial factors to HIV disease progression. *Ann Behav Med* 18, 30-39.

- Paul, D., Griffiths, 2006. CMV as a cofactor enhancing progression of AIDS. *J Clin Virol* 35, 489-492.
- Perna F.M., LaPerriere, A., Klimas, N., Ironson, G., Perry, A., Pavone, J., Goldstein, A., Majors, P., Makemson, D., Talutto, C., Schneiderman, N., Fletcher, M.A., Meijer, O.G., Koppes, L., 1999. Cardiopulmonary and CD4 cell changes in response to exercise training in early symptomatic HIV infection. *Med Sci Sports Exerc* 31, 973-979.
- Perry, S., Fishman, B., Jacobsberg, L., Frances, A., 1992. Relationships over 1 year between lymphocyte subsets and psychosocial variables among adults with infection by human immunodeficiency virus. *Arch Gen Psychiatry* 49, 396-401.
- 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 Behav Immun* 19, 325-333.
- 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 Behav Immun* 20, 279-289.
- Pothoff, G., Wasserman, K., Ostmann, H., 1994. Impairment of exercise capacity in various groups of HIV-infected patients. *Respiration* 61, 80-85.
- Poulsen, A.G., Aaby, P., Larsen, O., Jensen, H., Nauc ler, A., Lisse, I.M., Christiansen, C.B., Dias, F., Melbye, M., 1997. 9-year HIV-2-associated mortality in an urban community in Bissau, West Africa. *Lancet* 349, 911-914.
- Presanis, A.M., Gill, O.N., Chadborn, T.R., Hill, C., Hope, V., Logan, L., Rice, B.D., Delpech, V.C., Ades, A.E., De Angelis, D., 2010. Insights into the rise in HIV infections, 2001 to 2008: a Bayesian synthesis of prevalence evidence. *AIDS* 24, 2849-2858.
- Pressman, S.D., Cohen, S., Miller, G.E., Barkin, A., Rabin, B.S., Treanor, J.J., 2005. Loneliness, social network size, and immune response to influenza vaccination in college freshmen. *Health Psychol* 24, 297-306.
- Proske, U., Morgan, D.L., 2001. Muscle damage from eccentric exercise: mechanism, mechanical signs, adaptation and clinical applications. *J Physiol* 537, 333-345.
- Ramon, G., 1924. Sur la toxine et surranatoxine diphtheriques. *Ann Inst Pasteur* 33, 469-478.
- Ram rez-Marrero, F.A., Smith, B.A., Melendez-Brau, N., Santana-Bagur, J.L., 2004. Physical and leisure activity, body composition, and life satisfaction in HIV-positive Hispanics in Puerto Rico. *J Assoc Nurses AIDS Care* 15, 68-77.

- Rigsby, L.W., Dishman, R.K., Jackson, A.W., Maclean, G.S., Raven, P.B., 1992. Effects of exercise training on men seropositive for the human immunodeficiency virus-1. *Med Sci Sports Exerc* 24, 6-12.
- Rivas, P., Herrero, M.D., Puente, S., Ramirez-Olivencia, G., Soriano, V., 2007. Immunizations in HIV-infected adults. *AIDS Rev* 9, 173-187.
- Rodriguez-Barradas, M.C., Groover, J.E., Lacke, C.E., Gump, D.W., Lahart, C.J., Pandey, J.P., Musher, D.M., 1996. IgG antibody to pneumococcal capsular polysaccharide in human immunodeficiency virus-infected subjects: Persistence of antibody in responders, revaccination in nonresponders, and relationship of immunoglobulin allotype to response. *J Infect Dis* 173, 1347-1353.
- Rogers, C.J., Zaharoff, D.A., Hance, K.W., Perkins, S.N., Hursting, S.D., Schlom, J., Greiner, J.W., 2008. Exercise enhances vaccine-induced antigen-specific T cell responses. *Vaccine* 26, 5407-5415.
- Schroecksnadel, K., Sarcletti, M., Winkler, C., Mumelter, B., Weiss, G., Fuchs, D., Kemmler, G., Zangerle, R., 2008. Quality of life and immune activation in patients with HIV-infection. *Brain Behav Immun* 22, 881-889.
- Segerstrom, S.C., Schipper, L.J., Greenberg, R.N., 2008. Caregiving, repetitive thought, and immune response to vaccination in older adults. *Brain Behav Immun* 22, 744-752.
- Silberman, D.M., Wald, M.R., Genaro, A.M., 2003. Acute and chronic stress exert opposing effects on antibody responses associated with changes in stress hormone regulation of T-lymphocyte reactivity. *J Neuroimmunol* 144, 53-60.
- Smit, E., Crespo, C.J., Semba, R.D., Jaworowicz, D., Vlahov, D., Ricketts, E.P., Ramirez-Marrero, F.A., Tang, A.M., 2006. Physical activity in a cohort of HIV-positive and HIV-negative injection drug users. *AIDS Care* 18, 1040-1045.
- Smith, D.M., Strain, M.C., Frost, S.D.W., Pillai, S.K., Wong, J.K., Wrin, T., Liu, Y., Petropoulos, C.J., Daar, E.S., Little, S.J., Richman, D., 2006. Lack of neutralizing antibody response to HIV-1 predisposes to superinfection. *Virology* 355, 1-5.
- Smith, T.P., Kennedy, S.L., Fleshner, M., 2004. Influence of age and physical activity on the primary in vivo antibody and T cell-mediated responses in men. *J Appl Physiol* 97, 491-498.
- Solano, L., Costa, M., Salvati, S., Coda, R., Aiuti, F., Mezzaroma, I., Bertini, M., 1993. Psychosocial factors and clinical evolution in HIV-1 infection: A longitudinal study. *J Psychosom Res* 37, 39-51.
- Solomon, G.F., 1969. Emotions, stress the central nervous system, and immunity. *Ann N Y Acad Sci* 164, 335-343.

- Suzuki, K., Tagami, K., 2005. Voluntary wheel-running exercise enhances antigen-specific antibody-producing splenic B cell response and prolongs IgG half-life in the blood. *Eur J Appl Physiol* 94, 514-519.
- Terry, L., Sprinz, E., Ribeiro, J.P., 1999. Moderate and high intensity exercise training in HIV-1 seropositive individuals: A randomized trial. *Int J Sports Med* 20, 142,146.
- Tkachuk, G.A., Martin, G.L., 1999. Exercise therapy for patients with psychiatric disorders: Research and clinical implications. *Prof Psychol-Res Pr* 30, 275-282.
- 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. *Lancet* 353, 627-631.
- Wang, J., Song, H., Tang, X., Yang, Y., Vieira, V.J., Niu, Y., Ma, Y., 2011. Effect of exercise training intensity on murine T-regulatory cells and vaccination response. *Scand J Med Sci Sports*
- Whetten, K., Reif, S., Whetten, R., Murphy-McMillan, L.K., 2008. Trauma, Mental health, distrust, and stigma among HIV-positive persons: Implications for effective care. *Psychosom Med* 70, 531-538.
- Willoughby, D.S., McFarlin, B., Bois, C., 2003. Interleukin-6 expression after repeated bouts of eccentric exercise. *Int J Sports Med* 24, 15,21.
- Wood, P.G., Karol, M.H., Kusnecov, A.W., Rabin, B.S., 1993. Enhancement of antigen-specific humoral and cell-mediated immunity by electric footshock stress in rats. *Brain Behav Immun* 7, 121-134.
- Woods, J.A., Keylock, K.T., Lowder, T.W., Vieira, V.J., Zelkovich, W., Dumich, S., Colantuano, K., Lyons, K., Leifheit, K.A., Cook, M., Chapman-Novakofski, K., McAuley, E., 2009. Cardiovascular exercise training extends influenza vaccine seroprotection in sedentary older adults: The immune function intervention trial. *Am J Chin Med* 57, 2183-2191.

## CHAPTER TWO

### VACCINATION RESPONSE FOLLOWING AEROBIC EXERCISE: CAN A BRISK WALK ENHANCE ANTIBODY RESPONSE TO PNEUMOCOCCAL AND INFLUENZA VACCINATIONS?

#### ABSTRACT

High intensity acute exercise at the time of vaccination has been shown to enhance the subsequent antibody response. This study examines whether an acute moderate intensity aerobic intervention prior to vaccination can enhance antibody response to pneumonia and half dose influenza vaccination. Sixty young (age (SD) = 22.0 (6.1) yrs) and 60 older (age (SD) = 57.5 (6.5) yrs) adults attended the laboratory on two separate occasions. At the first session, baseline antibody titres were determined, before participants completed either a brisk walk around campus at >55% of their age-predicted heart rate maximum, or a resting control condition, for 45 minutes. After the intervention, all participants received a full-dose pneumococcal vaccination and a half-dose influenza vaccination. Four weeks later, participants returned for a follow up blood sample. Multivariate ANOVA revealed an increase in total antibody titres against the influenza vaccine ( $F_{(12, 106)} = 25.76, p <.001, \eta^2 = .75$ ) and both the IgM ( $F_{(12, 106)} = 17.10, p <.001, \eta^2 = .66$ ) and IgG ( $F_{(12, 106)} = 25.76, p <.001, \eta^2 = .75$ ) antibody titres against the pneumococcal vaccine. However, there were no significant Time  $\times$  Group interactions ( $p$ 's all  $>.15$ ), indicating that a 45 minute brisk walk prior to vaccination did not affect antibody response to either the influenza or pneumonia vaccine. The results suggest that higher intensity exercise is necessary to augment antibody response to vaccination.

## INTRODUCTION

Although vaccinations are highly effective public health measures, they do not always generate a robust and protective immune response (Ehreth, 2003; Murasko et al, 2002). Chemical adjuvants are added to the vaccine to help elicit a greater antibody response than the antigen alone (Ramon, 1924). Recent research has demonstrated that some behaviours, such as exercise, can induce endogenous adjuvants that enhance vaccination response (Edwards et al, 2006a; 2007; 2008; 2010). For example, 45 minutes of aerobic exercise, which involved an incremental test and then steady state exercise at 55% predicted maximum workload, significantly improved antibody responses to the influenza vaccination in women (Edwards et al, 2006a), and to meningitis A+C vaccine in men (Edwards et al, 2008), compared to non-exercising control groups. Similarly, a bout of eccentric resistance exercise of the biceps brachii and deltoid muscles also enhanced antibody response to influenza vaccination (Edwards et al, 2007; 2010). For eccentric exercise, recent research has demonstrated that this effect appears independent of exercise intensity. Edwards et al (2010) found that, although eccentric exercise was effective compared to control, no dose-response relationship was seen between the weight used (60%, 85%, or 110% of the individual's one repetition maximum) and the extent of the subsequent antibody response. In light of these findings, we tested whether an acute moderate intensity aerobic intervention would be capable of replicating the enhancing effects obtained with high intensity aerobic exercise.

These behavioural adjuvant studies all involved young healthy participants who have a relatively robust antibody response to vaccination (Edwards et al, 2006a; 2007; 2008; 2010). These studies found that augmentation of antibody response occurred only in less immunogenic strains, which elicited a relatively poor response in the control group, or where a half dose vaccination was given. It is possible that a ceiling effect may be present in these

young populations, whereby already robust antibody responses are not further improved by the intervention. This raises the possibility that populations which show reduced antibody responses to vaccination, such as older adults (Grubeck-Loebenstein et al, 2009), may benefit more from an exercise intervention than more immunocompetent populations. To address this question, the current study compares the efficacy of moderate intensity aerobic exercise in younger versus older adults.

As well as testing different populations, it is also useful to investigate the effects of the exercise on different types of vaccination. Vaccines against protein antigens, such as influenza, induce a thymus-dependent antibody response, whereas vaccines against bacterial polysaccharide antigens, such as *Streptococcus pneumoniae*, stimulate antibody responses independently of T helper lymphocytes. Previous exercise and stress research has revealed that both thymus dependent and independent vaccinations are susceptible to behavioural influences (Edwards et al, 2008; Burns et al, 2003; 2010). By examining how these vaccine types are affected by acute exercise, we may be able to determine which immune pathways are involved in the immunoenhancing effects of acute aerobic exercise. Furthermore, the study examines both of IgM and IgG responses to the pneumococcal vaccine to determine whether physical activity affects initial or long-term generation of antibodies.

The current study examined whether acute moderate intensity aerobic exercise prior to vaccination enhances antibody response to pneumonia and half dose influenza vaccination. A brisk walk, involving both up- and down-hill sections, was selected as the intervention, as it is accessible to older adults (Rippe et al, 1988). Further, it could be relatively easily introduced in a clinical setting, compared to cycling or resistance exercise which requires more equipment and supervision. The study compared the responses of a young and older participant cohort to determine if the efficacy of this intervention differs between the age



groups. It was predicted that the efficacy of the intervention on antibody response, measured 4 weeks post vaccination, will be greater for the older participants, as compared to the young cohort.

## **METHODS**

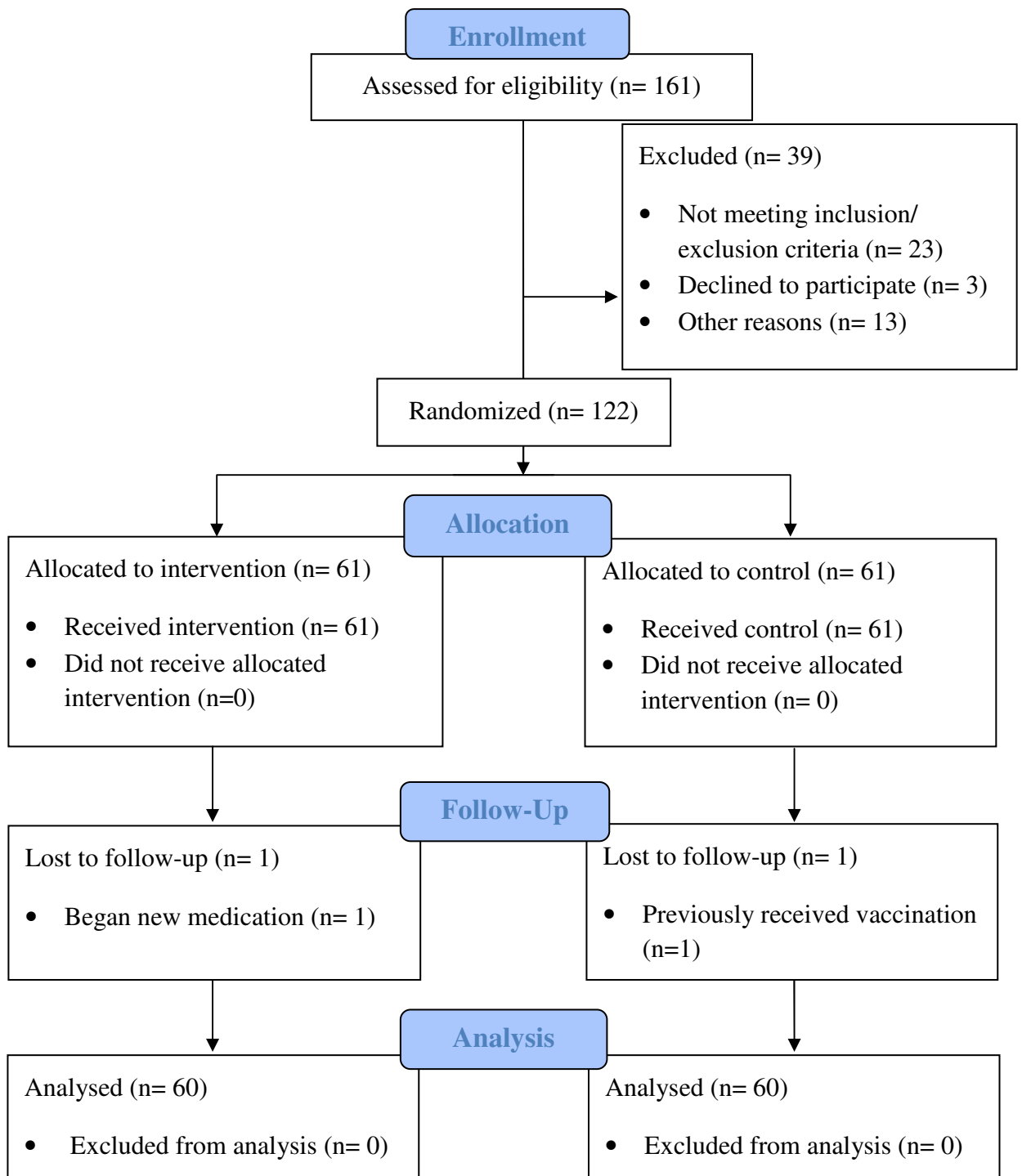
### **Participants**

One hundred and twenty two participants (see Figure 1 for CONSORT diagram) were recruited from The University of Birmingham campus and the West Midlands area via local newspapers, flyers, email and intranet advertisements, local community group visits, and by word of mouth (see Table 1 for basic group demographics). Prospective participants completed a telephone screening interview to check eligibility against the following criteria: either 18-30 or 50-64 years old; not highly endurance trained; no vaccination against influenza in the previous year or pneumonia in the last 10 years; no allergy to egg or chicken proteins; no latex allergy; no history of cancer, inflammatory disease or cardiovascular disease; no chronic obstructive pulmonary disorder, diabetes mellitus, asthma, congestive heart failure, Guillain Barre syndrome or psychiatric disorder; no females who were pregnant or breastfeeding; no current infection; and no medication or medical condition which may influence immune function. The 122 participants (60 young and 62 older adults) were randomised into 2 groups, with an equal number of males and females in each group. All participants gave written informed consent and the study was approved by the South Birmingham Local Research Ethics Committee.

### **Procedure**

Participants attended the laboratory on two separate occasions one month apart. At the first session, participants were pre-screened for contraindications to participation. They then completed a set of questionnaires, gave a blood sample, and height and weight were measured to calculate body mass index (BMI). Participants then underwent their allocated intervention

**Figure 1:** CONSORT flow diagram of participant retention.



	18-30 yrs cohort		50-64 yrs Cohort	
	Intervention group	Control Group	Intervention group	Control Group
Age (years)	21.37(2.82) <sup>†</sup>	21.13(2.19) <sup>†</sup>	57.94(4.40) <sup>†</sup>	58.55(4.38) <sup>†</sup>
BMI	25.12 (5.11)	23.82 (3.35)	25.74 (3.03)	26.21 (5.21)
On medication	13.3% <sup>†</sup>	3.3% <sup>†</sup>	38.7% <sup>†</sup>	25.8% <sup>†</sup>
Ethnicity (% white)	86.7%	93.3%	96.8%	93.5%
Walking Behaviour (min/week)	232.41 (153.29)*	363 (207.37)*	228.71 (219.95)*	282.09 (276.97)*
IPAQ Vigorous MET minutes/week	21.89 (12.01) <sup>†</sup>	24.13 (12.19) <sup>†</sup>	10.57 (13.13) <sup>†</sup>	12.27 (12.80) <sup>†</sup>
IPAQ Moderate MET minutes/week	14.17 (10.38)	12.00 (10.01)	10.75 (10.62)	10.50 (10.17)
% Smoked	0% <sup>†</sup>	0% <sup>†</sup>	6.5% <sup>†</sup>	6.5% <sup>†</sup>
Units of alcohol drank per month:				
None	14.3% <sup>†</sup>	6.7% <sup>†</sup>	19.4% <sup>†</sup>	22.6% <sup>†</sup>
1-5	57.1% <sup>†</sup>	70% <sup>†</sup>	74.2% <sup>†</sup>	74.2% <sup>†</sup>
6-10	25% <sup>†</sup>	20% <sup>†</sup>	3.2% <sup>†</sup>	3.2% <sup>†</sup>
11-20	3.6% <sup>†</sup>	3.3% <sup>†</sup>	3.2% <sup>†</sup>	NA <sup>†</sup>

**Table 1. Table of basic group demographics at baseline.**

Analysis of Variance and Chi squared tests were used to test for significant differences between groups and age cohorts. \*indicates a significant main effect of group. † indicates a significant main effect of age cohort. There were no interactions.

or control condition before receiving a full dose pneumonia vaccine (Pneumovax II, Sanofi Pasteur, batch number NL30250) and half dose influenza vaccination<sup>1</sup> (Fluarix, GSK, batch number AFLUA538AA) by a nurse. Following vaccination, participants remained in the laboratory for 15 minutes to monitor for immediate hypersensitivity reactions. Twenty-four to forty-eight hours after vaccination, participants were contacted via telephone to answer a short questionnaire regarding adverse events (no immediate hypersensitivity reactions or serious adverse events occurred). Four weeks later, participants returned to the laboratory to give a follow up blood sample. Upon completion of the study, participants received £30 or course research hours.

### **Intervention and control group**

Participants in the intervention group wore a heart rate monitor throughout the walk; average heart rate was used as a marker of physical activity intensity. After completing a warm-up lap and a series of lower body isometric muscle stretches, participants were instructed to walk at a brisk pace for 45 minutes around a specified route while maintaining their heart rate at or above 55% of their heart rate maximum, calculated as 220 minus age (Fox et al, 1971). The walking route included sections of uphill and downhill walking to elicit both concentric and eccentric contractions of the muscles. After the intervention, participants carried out a cool-down involving isometric muscle stretches. Those in the control group sat quietly in the laboratory for 45 minutes prior to receiving the vaccinations.

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<sup>1</sup> As with previous studies, a reduced dose (50%) influenza vaccination was administered, as healthy participants tend to elicit robust immune responses.

## **Health behaviour and physical activity questionnaires**

Health behaviours (smoking, alcohol consumption) were assessed using a questionnaire adapted from the Whitehall II study (Marmot et al., 1991). The Neighbourhood Physical Activity Questionnaire (NPAQ) (Giles-Corti et al, 2006) assessed the frequency and duration of recreational and transport-related walking within and outside the participants' neighbourhood. A modified version of the short-form International Physical Activity Questionnaire (IPAQ) (<http://www.ipaq.ki.se>) measured self-reported physical activity within the last 7 days. Items assessing walking behaviour were omitted to avoid duplication with the NPAQ. Vigorous and moderate metabolic equivalent of the task (MET) minutes and sitting behaviour were calculated as per the standard instructions (<http://www.ipaq.ki.se>).

## **Blood sampling and antibody status**

### **Blood sampling**

Blood samples were taken by venepuncture from the antecubital vein in the arm. Serum was collected in a 10ml plain red tube (BD Vacutainer, UK), was allowed to clot at room temperature for 1 hour, and then centrifuged at 3000rpm for 5 minutes. The separated serum was frozen at  $-20^{\circ}\text{C}$  for later antibody titre determination.

### **Pneumococcal Assay**

Luminex technology was used to assess twelve pneumococcal (Pn) IgG antibody serotypes (types 1, 3, 4, 5, 6b, 7f, 9v, 14, 18c, 19a, 19f, 23f) of the 23 contained in the pneumococcal vaccine. Further details of this assay are described elsewhere (Ferraro et al., 2007). Acquisition software (BioPlex Software Manager (version 4, BioRad, Labs, CA, USA) was

used to generate serotype antibody concentrations from a five parameter logistic curve fit. Serum Pn IgG and IgM levels are reported in µg/ml. Our data indicated that the intra and inter assay coefficient of variability were 6.5% and 9.8% respectively.

Serum samples that yielded values above the standard curve were re-run at a lower dilution of 1:500. If, following this further assay, values were still out of range above the curve, they were assigned the value of the top of the standard curve. Any values which were out of range below the standard curve were assigned the value of 0.01.

### **Influenza Assay**

Anti-influenza antibody titres were measured using an in-house haemagglutination inhibition test as described in the WHO Manual for Animal Influenza Diagnosis and Surveillance (World Health Organisation; <http://www.who.int>). The 2010-2011 influenza vaccine (Fluarix, GSK) contained three viral strains: A/California/7/2009, B/Brisbane/60/2008 and A/Perth/16/2009 derived from like antigen A/Victoria/210/2009. Details of this assay method have been described in detail elsewhere (Hirst et al, 1942; Pedersen, 2008).

### **Statistical Analysis**

Analyses were carried out using SPSS version 19.0 (SPSS Inc, Chicago, IL). Differences between groups and age cohorts at baseline were analysed using one way analysis of variance (ANOVA) for continuous data and Chi squared test for nominal variables.

Due to the skewed distribution of the antibody data, IgG and IgM pneumococcal and influenza antibody titres were subject to  $\log^{10}$  transformation for each strain. Multivariate repeated measures ANOVA was used to investigate antibody titres between baseline and 4

weeks post vaccination. Antibody responses to each pneumococcal strain (pn1, pn3, pn4, pn5, pn6b, pn7f, pn9v, pn14, pn18c, pn19a, pn19f, pn23f) were entered together as the dependent variables and Group (intervention, control) and Age (18-30 years, 50-64 years) cohort were entered together as the between subjects factors.

Participants were also classified according to whether they had achieved a clinically protective response to the influenza and pneumococcal vaccinations. For the influenza vaccination, responders were defined as those who achieved a four fold increase in influenza antibody titre 4 weeks after vaccination (Levine et al, 1987). Protective pneumococcal response was defined by achieving an absolute antibody level of 0.35 $\mu$ l/L for at least 8/12 pneumococcal strains (WHO, 2005). Chi squared tests were used to examine the differences between number of responders in each group.

Linear regression analyses were used to predict whether heart rate, percentage of heart rate, distance or walking speed during the intervention significantly predicted antibody response determined as the geometric mean of the 12 pneumococcal titres (pn1, pn3, pn4, pn5, pn6b, pn7f, pn9v, pn14, pn18c, pn19a, pn19f, pn23f) or influenza antibody titre (A/California, B/Brisbane, A/Perth) at follow up. Baseline antibody titre was entered at step one, followed by the demographic variables (sex, medication, smoker, drinker, vigorous MET minutes of physical activity, moderate MET minutes of physical activity, walking behaviour), in turn, at step two. If variables were significant, they were entered at step two, before entering the intervention variable (target heart rate, actual average heart rate, heart rate percentage, distance and speed) at step three.



## RESULTS

### Participants

Baseline group demographics are shown in Table 1; significant differences between age cohorts showed that older participants were more likely to be medicated ( $\chi^2 = 10.71, p <.01$ ), more likely to smoke ( $\chi^2 = 4.00, p =.05$ ), drank less alcohol ( $\chi^2 = 12.50, p =.01$ ) and took part in fewer vigorous MET minutes of physical activity per week ( $F_{(1, 120)} = 25.96, p <.001, \eta^2 = .18$ ), compared to the younger participants<sup>2</sup>. There was also a significant difference in walking behaviour between the intervention and control groups, where the control group reported more minutes of walking per week.

### Cardiac responses to intervention

Table 2 shows the cardiac responses to the intervention in the young and old intervention groups. There was a significant difference between age cohorts; as expected, older adults had a lower 55% age predicted heart rate maximum ( $F_{(1, 59)} = 1134.29, p <.001, \eta^2 = .95$ ) and actual average heart rate during the intervention ( $F_{(1, 59)} = 4.60, p =.04, \eta^2 = .07$ ). They also achieved a higher percentage of heart rate maximum, compared to the younger group ( $F_{(1, 59)} = 21.25, p <.001, \eta^2 = .27$ ). However, speed ( $F_{(1, 59)} = 2.71, p =.10, \eta^2 = .04$ ) and distance walked ( $F_{(1, 59)} = 2.94, p =.09, \eta^2 = .05$ ) were not significantly different between age cohorts.

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<sup>2</sup> When the multivariate ANOVA was repeated with each of these measures as a covariate, all Group and Interaction effects remained broadly the same.

	Intervention group(18- 30yrs)	Intervention group (50-64yrs)
Target heart rate during exercise (bpm)	109.17(1.80) <sup>†</sup>	89.30(2.72) <sup>†</sup>
Actual heart rate during exercise (bpm)	122.30(13.85) <sup>†</sup>	115.13(13.92) <sup>†</sup>
Actual % of maximum heart rate achieved	61.59(6.99) <sup>†</sup>	71.17(8.99) <sup>†</sup>
Walking speed (km/hr)	5.48(0.60)	5.26(0.58)
Distance (km)	4.10(0.43)	3.91(0.43)

**Table 2. Cardiovascular responses to the intervention**

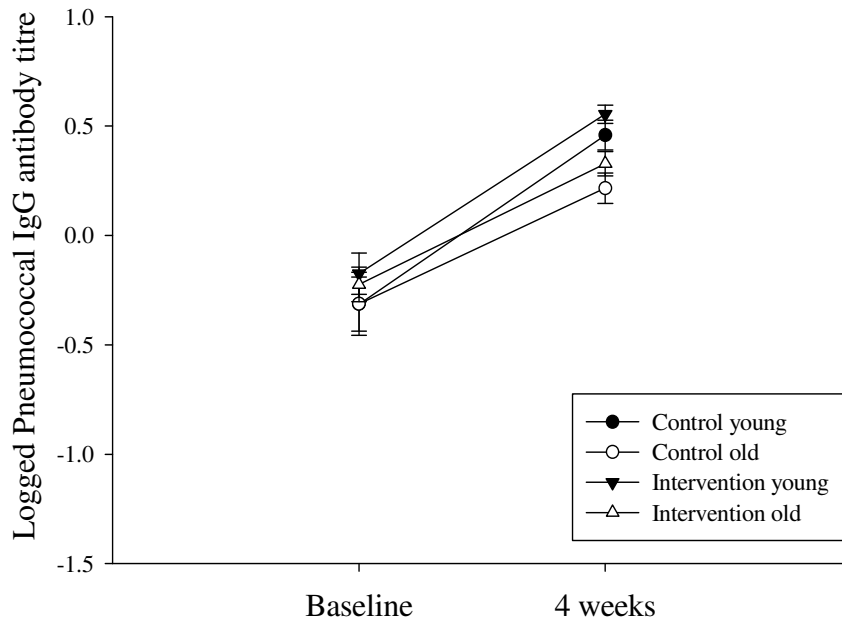
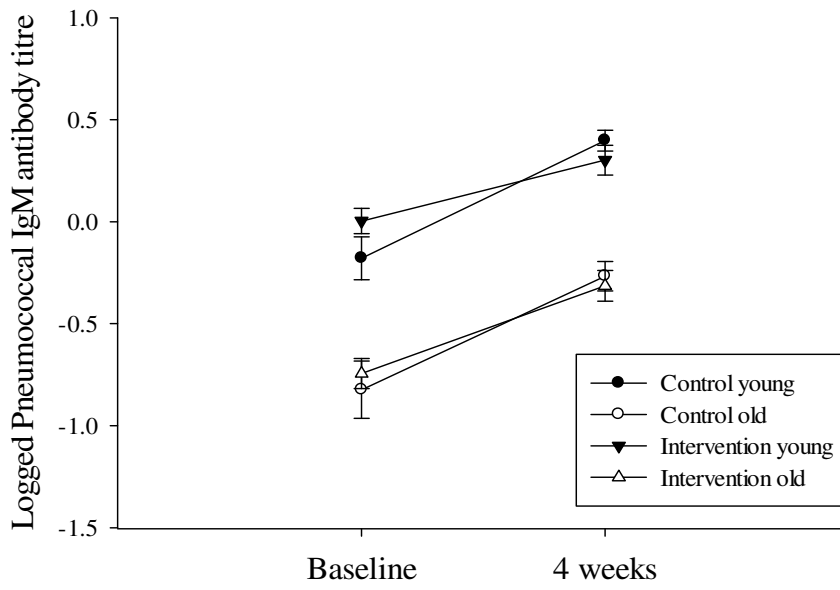
Analysis of Variance was used to test for significant differences between age cohorts. <sup>†</sup> indicates a significant main effect of age cohort.

## Antibody response to pneumococcal vaccination

Figures 2A and B show the average of the 12 logged IgM and IgG pneumococcal antibody responses from baseline to 4 week follow up. Repeated measures multivariate ANOVA, with pneumococcal IgM responses to all 12 strains entered as dependent variables, revealed a significant overall increase in antibody titre ( $F_{(12, 106)} = 17.10, p < .001, \eta^2 = .66$ ), but no significant Time  $\times$  Group interaction ( $F_{(12, 106)} = 1.45, p = .16, \eta^2 = .14$ ). There was a significant main effect of Age ( $F_{(12, 106)} = 7.98, p < .001, \eta^2 = .48$ ), and Time  $\times$  Age interaction ( $F_{(12, 106)} = 2.38, p < .01, \eta^2 = .21$ ), in which older adults had significantly lower antibody titres overall, and a poorer antibody response to the vaccine, compared to the younger adults. When examining the univariate ANOVA results for each individual strain, 11 of the 12 strains showed a significant increase in time ( $p$ 's all  $< .001$ ; for Pn3  $p = .84$ ). Three strains (pn1, pn4, pn18c) had a significant Time  $\times$  Group interaction ( $p$ 's all  $< .05$ ), which indicated that for these strains the control group had significantly higher antibody responses, compared to the intervention group. One strain (pn3) showed a significant Time  $\times$  Age interaction ( $F_{(1, 119)} = 14.63, p < .001, \eta^2 = .16$ ); young participants had decreased, whereas older participants showed increased pn3 antibody titres over the course of the intervention.

Pneumococcal IgG titres were also analysed using repeated measures multivariate ANOVA, which revealed that antibody titres significantly increased over the study period ( $F_{(12, 106)} = 25.76, p < .001, \eta^2 = .75$ ), but there was no significant Time  $\times$  Group interaction ( $F_{(12, 106)} = 0.63, p = .81, \eta^2 = .07$ ). There was a significant between subjects effect of Group ( $F_{(12, 106)} = 1.90, p = .04, \eta^2 = .18$ ) and Age ( $F_{(12, 106)} = 5.58, p < .001, \eta^2 = .39$ ); those in the intervention group had marginally higher antibody titres overall, compared to the control group, and older adults had significantly lower antibody titres, compared to the younger participants. However, there was no significant Time  $\times$  Age interaction ( $F_{(12, 106)} = 0.86, p = .59, \eta^2 = .10$ ).

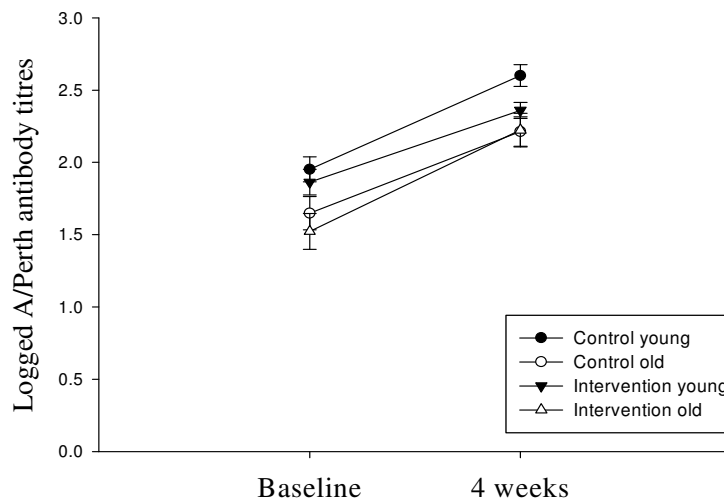
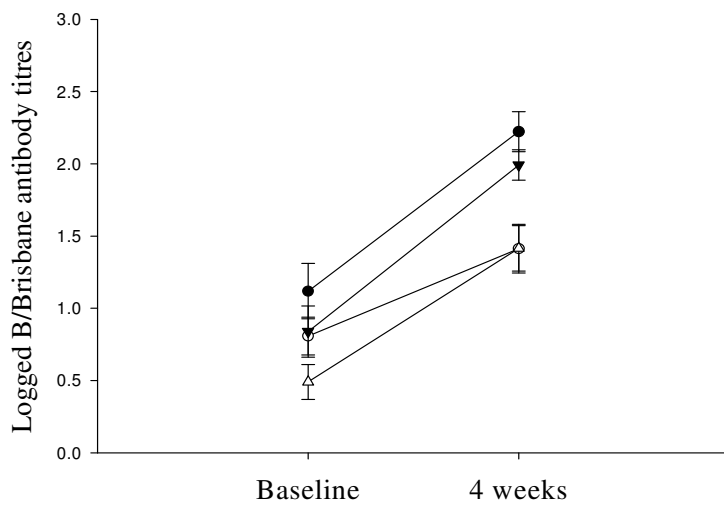
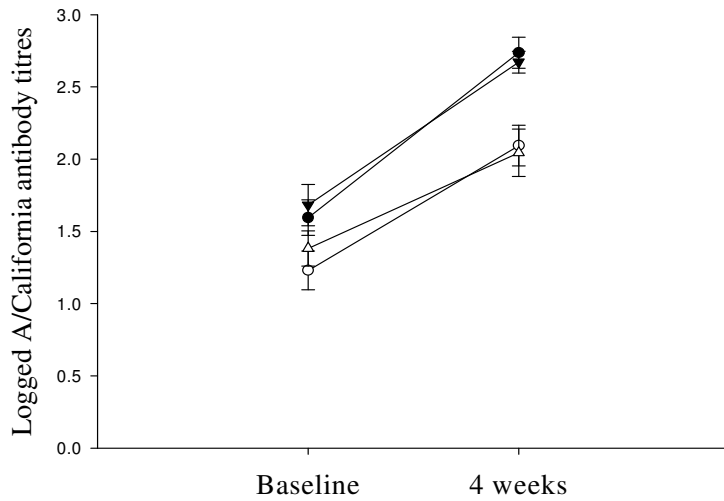
The univariate analyses showed that all 12 strains significantly increased over time ( $p$ 's all  $<.001$ ), but no strains showed any significant Time  $\times$  Group interactions ( $p$ 's all  $>.17$ ). Univariate data also showed a significant Time  $\times$  Age interaction, where young participants had a greater increase in pn9v ( $F_{(1, 119)} = 4.33, p = .04, \eta^2 = .04$ ) and pn23f ( $F_{(1, 119)} = 4.24, p = .04, \eta^2 = .04$ ) antibody titres, compared to the older cohort. There was also no difference between groups in terms of the number of responders to the IgG ( $\chi^2 = 0.49, p = .57$ ) or IgM pneumococcal vaccination ( $\chi^2 = 0.23, p = .67$ ).



**Figure 2A and B:** Mean (SE) of the 12 IgM and IgG logged pneumococcal strains with standard error from baseline to 4 weeks post vaccination between young (filled) and old (open) intervention (triangles) and control (circles) groups.

### **Antibody response to influenza vaccination**

Figures 3A, B and C show the logged antibody titres at baseline and follow up for the A/California, B/Brisbane and A/Perth influenza strains. Multivariate repeated measures ANOVA revealed that influenza titres significantly increased between baseline and follow up ( $F_{(3, 114)} = 110.10, p < .001, \eta^2 = .74$ ), but there were no significant Time  $\times$  Group interactions ( $F_{(3, 114)} = 1.54, p = .21, \eta^2 = .04$ ). There was a significant main effect of Age ( $F_{(3, 114)} = 11.76, p < .001, \eta^2 = .24$ ) and Time  $\times$  Age interaction ( $F_{(3, 114)} = 3.76, p = .01, \eta^2 = .09$ ), which again showed that older adults had significantly lower antibody titres, and responses, compared to the younger cohort. The univariate results showed that all three strains increased over time ( $p$ 's all  $< .001$ ), but none showed a significant Time  $\times$  Group interaction ( $p$ 's all  $> .19$ ). Furthermore, there was a significant Time  $\times$  Age interaction in the A/California ( $F_{(1, 116)} = 5.13, p = .03, \eta^2 = .04$ ) and B/Brisbane ( $F_{(1, 116)} = 6.05, p = .02, \eta^2 = .05$ ) strains, where younger adults achieved a higher antibody response, compared to the older adults. In addition, when comparing the number of fourfold responders to the influenza vaccine, results showed no significant differences between intervention and control groups for A/California ( $\chi^2 = 0.84, p = .46$ ), B/Brisbane ( $\chi^2 = 0.03, p = .50$ ), or A/Perth ( $\chi^2 = 0.35, p = .69$ ) influenza strains.



**Figure 3A, B and C:** Logged A/California, B/Brisbane and A/Perth influenza strain titres and standard error from baseline to 4 weeks post vaccination between young (filled) and old (open) intervention (triangles) and control (circles) groups.

## Predictors of follow up antibody response

Regression analyses, controlling for age, investigated whether any physical activity or intervention variables predicted antibody response. Baseline antibody titre was entered at step one of the regression, with the demographic variable (sex, medicated, smoker, drinker, vigorous MET minutes of physical activity, moderate MET minutes of physical activity, walking behaviour) at step two. If any variables emerged significant, they were controlled for, before entering the intervention variable at step three. Higher vigorous MET minutes of physical activity significantly predicted achieving a higher A/California  $\beta = .30$ ,  $t = 2.55$ ,  $p = .01$ ,  $\Delta R^2 = .091$  and also showed a trend toward predicting B/Brisbane  $\beta = .23$ ,  $t = 1.89$ ,  $p = .06$ ,  $\Delta R^2 = .051$  antibody response. Furthermore, age cohort significantly predicted antibody response for pneumococcal IgM  $\beta = -.39$ ,  $t = -.3.02$ ,  $p < .01$ ,  $\Delta R^2 = .081$ , IgG  $\beta = -.36$ ,  $t = -.3.19$ ,  $p < .01$ ,  $\Delta R^2 = .128$  and A/California  $\beta = -.35$ ,  $t = -.3.03$ ,  $p < .01$ ,  $\Delta R^2 = .119$  and B/Brisbane  $\beta = -.30$ ,  $t = -.2.55$ ,  $p = .01$ ,  $\Delta R^2 = .083$  influenza strains, where older participants had significantly lower antibody responses. No other demographic variable (smoking, moderate MET minutes of physical activity) predicted antibody response to pneumococcal IgG or IgM, or the influenza strains. After controlling for the relevant significant demographic variables, the intervention variables were entered at step three of the regression. Heart rate percentage during the intervention showed a trend toward significantly predicting follow up A/California titres,  $\beta = .25$ ,  $t = 1.79$ ,  $p = .08$ ,  $\Delta R^2 = .042$ , where a higher heart rate percentage predicted a higher antibody response. No other intervention variable (target heart rate, average heart rate, speed, distance, number of laps) predicted antibody response to any other of the vaccinations ( $p$ 's all  $> .14$ ).



## **DISCUSSION**

This study found that older participants had lower antibody responses to pneumococcal and half dose influenza vaccination, compared to younger adults. This suggests that the immune systems of 50-64 year old adults are already somewhat impaired, prior to reaching over 65 years old, at which immunosenescence is traditionally thought to become apparent (Goodwin et al, 2006). Contrary to our hypothesis, exercise did not augment the antibody response to vaccination in either age cohort. In fact, three pneumococcal IgM antibody strains (pn1, pn4, pn18c) showed reduced antibody titres in the intervention group. On the whole, no measures related to the intervention predicted antibody response.

These findings do not replicate our previous findings that a 45 minute bout of moderate cycling exercise prior to influenza vaccination improved antibody response to one of the influenza strains (Edwards et al, 2006a). There were two main differences between the previous acute exercise study and the one presented here, which may explain these discrepant findings. Firstly, this study was designed to be of a lower intensity. However, it is unlikely that this lower intensity explains the different results, as, in fact, both the young and older participants in the intervention group voluntarily exceeded their target intensity of 55% of their maximal percentage heart rate (achieving 61% and 71% respectively). This implies that the two studies had interventions of relatively similar workloads. However, the previous cycling task included a 16 min sub-maximal incremental stage, during which participants exercised at increasingly intense workloads, in order to calculate their individual predicted maximum. This protocol has been shown to induce interleukin-6 (IL-6) responses (Edwards et al, 2006b), which are typically not apparent in response to walking (Markovitch et al, 2008). As higher levels of IL-6 at time of vaccination have been shown to be associated with a greater antibody response to vaccination (Krakauer et al, 1995), this may explain why no

enhancement of antibody response was seen in our study. Secondly, our study employed a walking protocol, whereas the previous study used a cycle ergometer intervention. Walking is a habitual exercise for most people (Bassett et al, 2008), whereas cycling is less so. As immune responses have been shown to be more pronounced in response to unaccustomed exercise (Sorichter et al, 2006), this may help explain why an incremental cycling protocol may have a greater benefit for vaccination response than walking at a similar intensity.

The current study was designed to test an intervention that could be plausibly introduced into clinical practice, but did not focus specifically on determining mechanisms underpinning exercise-induced augmentation of antibody response to vaccination. However, evidence from some studies may explain why our intervention did not enhance antibody response. An increase in lymphatic drainage from contracting muscles is seen following exercise (Havas et al, 1997) and this consequently may enhance immune cell transportation to the lymph nodes (Swartz et al, 2008). Moreover, the demargination of immune cells into tissue and T and B cell lymphocytosis following exercise may also explain vaccination enhancement (Shek et al, 1995; Dhabhar et al, 1995). As exercise induced lymphocytosis is transient (Anane et al, 2009; Campbell et al, 2009; Turner et al, 2010) and our exercise intervention was relatively modest, it is possible that levels of circulating immune cells quickly returned to baseline levels and, as such, do not augment the antibody response to vaccination. A subsequent study is needed to investigate whether a vaccination *prior* to the acute intervention would have more beneficial effects on antibody response.

Despite no effect of the intervention, this study did show that older adults had significantly lower antibody responses to vaccination, compared to a younger cohort. This suggests that some age-related immunological changes may occur relatively early compared to previous reports (Goodwin et al, 2006). Previous studies have shown that some people in this age

bracket have begun to experience other age-associated declines in immune function, such as decreased number of naïve T and B lymphocytes and loss of costimulatory CD28 from effector T cells (Grubeck-Loebenstein et al, 2010; Vallejo, 2005), which could explain the reduced antibody response to vaccination.

There are some limitations to this study. Firstly, there was no direct measure of the participants' fitness levels, which was not, therefore, controlled in the 45 minute walk. Previous research has found that cardiovascular fitness is positively related to antibody response to vaccination (Keylock et al, 2007; Smith et al, 2004), which suggests that those who had a higher fitness level may not have achieved such a high average heart rate during the intervention. However, screening procedures ensured no participants were highly endurance trained and reported exercise measures did not significantly correlate with antibody response to vaccination. Furthermore, as participants needed to return to the laboratory following the walking intervention, there was a delay between cessation of exercise and vaccine administration. This may have allowed any exercise-induced immunological alterations to return to baseline and, as such, may explain the null results. Future research could seek to identify when is the best window of opportunity regarding immune function status following an acute bout of exercise.

In conclusion, a 45 minute brisk walk prior to pneumococcal and influenza vaccination did not improve antibody response. It is possible that moderate intensity walking is not sufficient as a physical stressor, or that the timing of the intervention in relation to the vaccination may not have been appropriate to affect antigen presentation or the generation of the antibody response to vaccination. However, it was found that older participants had a significantly lower antibody response to some of the vaccination strains, compared to the younger cohort,

suggesting that the development of effective behavioural adjuvants remains a priority, particularly for these more vulnerable populations.

## REFERENCES

- Anane, L.H., Edwards, K.M., Burns, V.E., Drayson, M.T., Riddell, N.E., van Zanten, J.J.C.S., Wallace, G.R., Mills, P.J., Bosch, J.A., 2009. Mobilization of CD8+ T lymphocytes in response to psychological stress, exercise, and beta agonist infusion. *Brain Behav Immun* 23, 823-829.
- Bassett D.R., Pucher, J., Buehler, R., Thompson, D. L., Crouter, S. E., 2008. Walking, cycling, and obesity rates in Europe, North America, and Australia. *J Phys Act Health* 5, 795-814.
- Burns, V.E., Carroll, D., Ring, C., Drayson, M., 2003. Antibody response to vaccination and psychosocial stress in humans: relationships and mechanisms. *Vaccine* 21, 2523-2534.
- Burns, V.E., Gallagher, S., 2010. Antibody response to vaccination as a marker of in vivo immune function in psychophysiological research. *Neurosci Biobehav Rev* 35, 122-126.
- Campbell, J.P., Riddell, N.E., Burns, V.E., Turner, M., Veldhuijzen van Zanten, J.C.S., Drayson, M.T., Bosch, J.A., 2009. Acute exercise mobilises CD8+ T lymphocytes exhibiting an effector-memory phenotype. *Brain Behav Immun* 23, 767-775.
- Dhabhar, F.S., Miller, A.H., McEwen, B.S., Spencer, R.L., 1995. Effects of stress on immune cell distribution. Dynamics and hormonal mechanisms. *J Immunol* 154, 5511-5527.
- 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 Behav Immun* 20, 159-168.
- Edwards, K.M., Burns, V.E., Ring, C., Carroll, D., 2006. Individual differences in the interleukin-6 response to maximal and submaximal exercise tasks. *J Sports Sci* 24, 855-862.
- Edwards, K.M., Burns, V.E., Allen, L.M., McPhee, J.S., Bosch, J.A., Carroll, D., Drayson, M., Ring, C., 2007. Eccentric exercise as an adjuvant to influenza vaccination in humans. *Brain Behav Immun* 21, 209-217.
- Edwards, K.M., Burns, V.E., Adkins, A.E., Carroll, D., Drayson, M., Ring, C., 2008. Meningococcal A vaccination response is enhanced by acute stress in men. *Psychosom Med* 70, 147-151.
- Edwards, K.M., Campbell, J.P., Ring, C., Drayson, M.T., Bosch, J.A., Downes, C., Long, J.E., Lumb, J.A., Merry, A., Paine, N.J., Burns, V.E., 2010. Exercise intensity does not influence the efficacy of eccentric exercise as a behavioural adjuvant to vaccination. *Brain Behav Immun* 24, 623-630.

- Ferraro, A., Drayson, M., Savage, C., MacLennan, I., 2008. Levels of autoantibodies, unlike antibodies to all extrinsic antigen groups, fall following B cell depletion with Rituximab. *Eur. J. Immunol.* 38, 292-298.
- Hirst, G. K., 1942. The quantitative determination of influenza virus and antibodies by means of red cell agglutination. *J Exp Med* 75, 49-64.
- Giles-Corti, B., Timperio, A., Cutt, H., Pikora, T.J., Bull, F.C.L., Knuiiman, M., Bulsara, M., Van Niel, K., Shilton, T., 2006. Development of a reliable measure of walking within and outside the local neighborhood: RESIDE's neighborhood physical activity questionnaire. *Prev Med* 42, 455-459.
- Goodwin, K., Viboud, C., Simonsen, L., 2006. Antibody response to influenza vaccination in the elderly: A quantitative review. *Vaccine* 24, 1159-1169.
- Grubeck-Loebenstien B, Della Bella, S., Iorio, A., Michel, J. P. Pawelec, G., Solana, R., 2009. Immunosenescence and vaccine failure in the elderly. *Aging-Clin Exp Res* 21, 201-209.
- Grubeck-Loebenstien, B., 2010. Fading Immune Protection in Old Age: Vaccination in the elderly. *J Comp Pathol* 142, S116-S119.
- Havas, E., Parviainen, T., Vuorela, J., Toivanen, J., Nikula, T., Vihko, V., 1997. Lymph flow dynamics in exercising human skeletal muscle as detected by scintigraphy. *J Physiol* 504, 233-239.
- Ehreth, J., 2003. The value of vaccination: a global perspective. *Vaccine* 21, 4105-4117.
- Keylock, K.T., Lowder, T., Leifheit, K.A., Cook, M., Mariani, R.A., Ross, K., Kim, K., Chapman-Novakofski, K., McAuley, E., Woods, J.A., 2007. Higher antibody, but not cell-mediated, responses to vaccination in high physically fit elderly. *J Appl Physiol* 102, 1090-1098.
- Krakauer, T., 1995. Levels of interleukin 6 and tumor necrosis factor in serum from humans vaccinated with live, attenuated *Francisella tularensis*. *Clin Diagn Lab Immunol* 2, 487-488.
- Levine M, Beattie, B. L., McLean, D. M., Corman, D., 1987. Characterization of the immune response to trivalent influenza vaccine in elderly men. *Am J Geriatr Soc* 35, 609-615.
- Markovitch, D., Tyrrell, R.M., Thompson, D., 2008. Acute moderate-intensity exercise in middle-aged men has neither an anti- nor proinflammatory effect. *J Appl Physiol* 105, 260-265.
- Marmot, M.G., Stansfeld, S., Patel, C., North, F., Head, J., White, I., Brunner, E., Feeney, A., Marmot, M.G., Smith, G.D., 1991. Health inequalities among British civil servants: the Whitehall II study. *Lancet* 337, 1387-1393.

- Murasko, D.M., Bernstein, E.D., Gardner, E.M., Gross, P., Munk, G., Dran, S., Abrutyn, E., 2002. Role of humoral and cell-mediated immunity in protection from influenza disease after immunization of healthy elderly. *Exp Gerontol* 37, 427-439.
- Pedersen, J.C., 2008. Hemagglutination-Inhibition Test for avian influenza virus subtype identification and the detection and quantitation of serum antibodies to the avian influenza virus. In: Spackman, E. (Ed.), Humana Press, pp.53-66.
- Ramon, G., 1924. Sur la toxine et surranatoxine diphtheriques. *Ann Inst Pasteur* 33, 469-478.
- Rippe, J.M., Ward, A., Porcari, J.P., Freedson, P.S., 1988. Walking for Health and Fitness. *JAMA* 259, 2720-2724.
- Shek, P.N., Sabiston, B.H., Buguet, A., Radomski, M.W., 1995. Strenuous exercise and immunological changes. *Int J Sports Med* 16, 466,474.
- Sorichter, S., Martin, M., Julius, P., Scwartz, A., Huonker, M., Luttmann, W., Walterspacher, S., Berg, A., 2006. Effects of unaccustomed and accustomed exercise on the immune response in runners. *Med Sci Sports Exerc* 38, 1739-1745.
- Swartz, M.A., Hubbell, J.A., Reddy, S.T., 2008. Lymphatic drainage function and its immunological implications: From dendritic cell homing to vaccine design. *Semin Immunol* 20, 147-156.
- Turner, J.E., Aldred, S., Witard, O.C., Drayson, M.T., Moss, P.M., Bosch, J.A., 2010. Latent Cytomegalovirus infection amplifies CD8 T-lymphocyte mobilisation and egress in response to exercise. *Brain Behav Immun* 24, 1362-1370.
- Vallejo, A.N., 2005. CD28 extinction in human T cells: altered functions and the program of T-cell senescence. *Immunol Rev* 205, 158-169.
- WHO., 2005. Recommendations for the production and control of pneumococcal conjugate vaccination.

## CHAPTER THREE

### A LIFESTYLE PHYSICAL ACTIVITY INTERVENTION AND THE ANTIBODY RESPONSE TO PNEUMOCOCCAL VACCINATION IN WOMEN

#### ABSTRACT

The present study investigates whether a lifestyle physical activity intervention can improve antibody response to vaccination. Eighty-nine sedentary women (Mean age (SD) = 47.4 (6.9) years) were recruited and randomised to an intervention (n = 44) or control (n = 45) group. Participants attended an initial familiarisation session, where they were given a sealed pedometer to assess baseline walking activity over one week. At the second session, body composition and aerobic fitness assessments were performed and questionnaires were completed. The intervention group then received a physical activity consultation, use of an unsealed pedometer, and weekly telephone/email prompting, whereas the control group received an advisory leaflet. Twelve weeks later, participants gave a blood sample before receiving a pneumococcal vaccination. A further four weeks later, antibody status, questionnaires, objective walking behaviour, body composition and aerobic fitness measurements were reassessed. Results showed a significant time  $\times$  group interaction for both objective ( $F_{(1, 67)} = 9.63, p < .01, \eta^2 = .13$ ) and self-reported walking behaviour ( $F_{(1, 68)} = 11.25, p < .01, \eta^2 = .14$ ); the intervention group increased both measures of walking behaviour, whereas the control group did not change. Multivariate ANOVA revealed an overall significant increase in pneumococcal titres ( $F_{(11, 61)} = 20.43, p < .01, \eta^2 = .79$ ). However, there was no significant time  $\times$  group interaction ( $F_{(11, 61)} = 0.90, p = 0.55, \eta^2 = .14$ ). Despite this, when all participants were included in the analysis, results showed that higher moderate MET



minutes of physical activity per week significantly predicted higher antibody responses at four weeks post vaccination,  $\beta = .22$ ,  $t = 2.43$ ,  $p = .02$ ,  $\Delta R^2 = .048$  and higher minutes of walking per week predicted a greater antibody response at 6 months post vaccination,  $\beta = .24$ ,  $t = 1.99$ ,  $p = .05$ ,  $\Delta R^2 = .057$ . In conclusion, only individual physical activity behaviour, and not participation in a lifestyle physical activity intervention, was associated with antibody response to pneumococcal vaccination in sedentary middle aged women.

## INTRODUCTION

Research conducted over the past 20 years accrued evidence for a 'J shaped' relationship between physical activity and immune function, whereby those who engaged in moderate levels of physical activity had fewer upper respiratory tract infections than those who were sedentary or taking part in vigorous levels of physical activity (Nieman, 1994). However, much of the supporting evidence is based on naturalistic infections (Nieman, 1993; Heath et al, 1991). While ecologically valid, such findings do not control for rates of antigen exposure and often rely on self report measures which do not necessarily confirm actual infection. Measuring antibody response to vaccination has been proposed as a more controlled method to examine the influence of behavioural factors, such as exercise, on *in vivo* immune function (Burns and Gallagher 2010). The vaccine model controls for exposure and yields antibody titre as an objective outcome measure.

Cross sectional studies have found a positive relationship between cardiovascular fitness and vaccination response (Keylock et al, 2007; Smith et al, 2004). However, related lifestyle factors, such as a healthy diet or smoking status, may confound this relationship between exercise and vaccination responses. These concerns are partly alleviated by supporting findings from intervention studies. Kohut et al (2004) and Grant et al (2008) found that those who completed a 10 month supervised exercise intervention prior to influenza or keyhole limpet hemocyanine (KLH) vaccination showed larger antibody responses, demonstrating that both primary (KLH) and secondary (influenza) antibody responses are affected by exercise status. Although these studies used very small samples (n= ~14 per group), a larger recent intervention (n= 144) also found a higher percentage of vaccination responders after a 10 month aerobic intervention, compared to a flexibility class control group (Woods et al, 2009). Although the mechanisms for this are not yet clear, previous research has shown that exercise

increases *ex-vivo* IFN- $\gamma$  in the elderly (Kohut et al, 2005) and that, at least *in vitro*, IFN- $\gamma$  concentrations are positively associated with antibody secreting B-cell frequencies in peripheral blood mononuclear cells taken from recipients of the hepatitis B vaccine (Bocher et al, 1999). There are, therefore, plausible biological pathways through which exercise may improve antibody responses to vaccination.

Although these highly structured and supervised exercise interventions have been effective, they would be difficult and costly to administer to large populations in the longer term. It is possible that a lifestyle orientated intervention, in which an individual is encouraged to integrate physical activity into their daily life, may be used as an alternate form of intervention that may be easier to implement in a clinical setting. One approach to increase lifestyle physical activity is the use of physical activity consultations, based on the trans-theoretical model of behaviour change (Prochaska and Diclemente, 1986). Consultations involve a semi-structured discussion about the reasons for and against physical activity participation, the risks of inactivity, awareness of opportunities to engage in physical activity, and the importance of social support, relapse prevention and goal setting opportunities. The consultation is tailored to suit the individual's needs and encourages them to take responsibility for their own behaviour change (Kirk et al, 2007). Women are often more readily recruited for these types of studies, which may be due to the higher prevalence of physical inactivity within this population (Martinez-Gonzalez et al, 2001; Azevedo et al, 2007) or that a more moderate physical activity behaviour, such as walking, is often a preferred exercise choice for women (Salmon et al, 2003). The use of pedometers and regular prompting are also successful tools for increasing physical activity behaviour and adherence (Bravata et al, 2007, Kazdin, 1989), and can be used in conjunction with the consultation to help increase physical activity levels (Baker et al, 2008). Kirk et al (2004) and Baker et al

(2008) found that physical activity counselling increased subjective and objective levels of physical activity over 6 and 4 months, respectively. Similarly, Dunn et al (1999) found comparable increases in cardiovascular fitness and self reported physical activity for both structured and non-structured interventions.

Lifestyle physical activity interventions are also associated with positive psychological outcomes, such as higher positive affect (Baker et al, 2008). Such psychological outcomes have shown a relationship with antibody response to vaccination. For example, low stress (Burns et al, 2003), low negative affect (Marsland et al, 2001) and high positive affect (Marsland et al, 2006) have been associated with a higher antibody response to vaccination. As some of the benefits of exercise interventions have been shown to be mediated by psychological factors (Kohut et al, 2005), this is a potential indirect pathway through which a lifestyle physical activity intervention could benefit antibody response to vaccination.

The majority of studies have focussed on the antibody response to influenza and KLH vaccination. These vaccines contain proteins that induce a thymus (T) dependent immune response, which requires both B cells and CD4+ T cells (Abbas and Lichtman, 2006). However, to our knowledge, no one has investigated the effect of habitual physical activity on antibody response to polysaccharide antigens that stimulate a T independent response. By examining the relative susceptibility of different types of vaccination, it is possible to start to elucidate which aspects of the *in vivo* immune response to antigen are most influenced by exercise (Phillips et al, 2005; Gallagher et al, 2008; Burns and Gallagher, 2010). Further, from a clinical perspective, it is important to understand how exercise influences protection against bacterial infections, such as pneumococcus, as they are a major source of morbidity and mortality, especially in older adults (Feldman, 1999; Marrie, 2000).

The present study examined whether a lifestyle physical activity intervention can affect antibody response to pneumococcal vaccination in women at both 4 weeks and 6 months post vaccination. It also evaluated physiological or psychological changes associated with the intervention, and whether these are related to the antibody response. It was hypothesised that those in the physical activity consultation group would have a higher antibody response at 4 weeks and 6 months post vaccination, compared to the control group, and that the extent of these changes would relate to the improvements seen in cardiovascular fitness and body composition.

## **METHODS**

### **Participants**

Ninety two women were recruited via local newspapers, flyers, email and intranet advertisements. Prospective participants completed a telephone screening interview to check eligibility against the following inclusion criteria: 35 to 65 years old; sedentary (participated in less than 30 minutes of moderate intensity exercise on five days of the week (Haskell et al, 2007)); no history of cancer, inflammatory disease or cardiovascular disease; no chronic obstructive pulmonary disorder, diabetes mellitus, asthma, congestive heart failure or psychiatric disorder; and no medication which interferes with immune responses. Eligible participants (n=89) were randomised into an intervention or educational control group; if participants from the same place of recruitment (e.g. workplace, community group) they were cluster randomised to maintain the integrity of the intervention. Table 1 presents a comparison of the two groups on demographic variables.

	Intervention group	Control Group
Age (years)	47.84 (SD = 6.56)	46.87 (SD = 7.38)
On medication (%)	22.7	15.6
Smoker (%)	6.8	11.1
<b>Ethnicity</b>		
White (%)	95.5	86.7
<b>Menopause status</b>		
Pre-menopause (%)	54.5	55.6
Post/Peri-menopause (%)	45.5	42.2
Did not answer (%)	0	2
<b>Occupation status</b>		
Non-manual (%)	97.7	88.9
Manual (%)	2.3	8.9
Did not answer (%)	0	2.2

**Table 1. Comparison of demographics variables.**

Chi square and univariate ANOVA tests revealed no significant differences between groups.

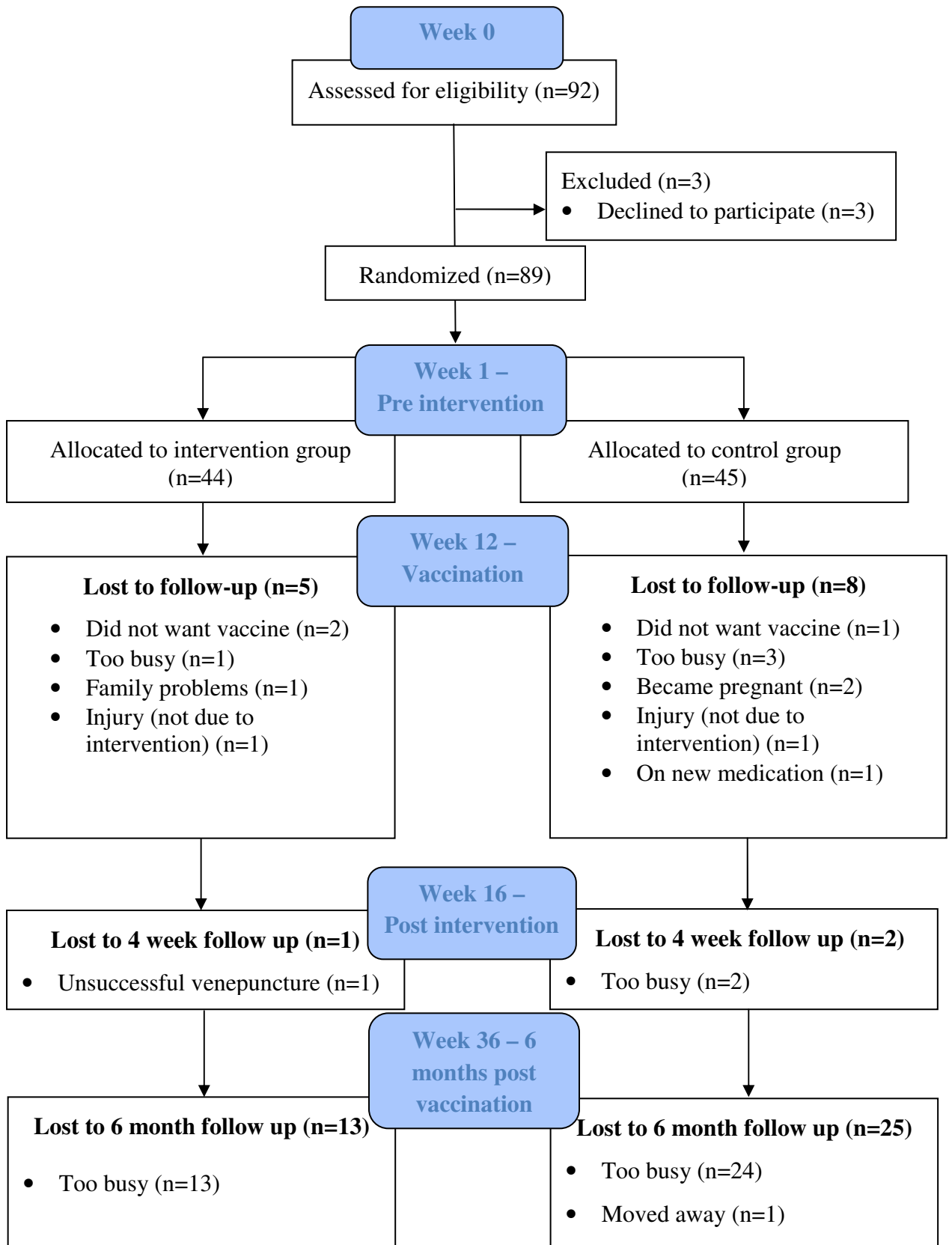
## **Procedure**

Participants attended the laboratory five times over nine months. The first visit involved familiarisation with the laboratory, giving informed consent, and completing a general health questionnaire. Participants were given a sealed pedometer to wear for one week to obtain a baseline assessment (total number of steps per week) of their lifestyle physical activity. At the second session, participants completed a battery of questionnaires and were assessed to determine body composition, blood pressure and aerobic fitness. They then received the intervention, as described below, according to their group allocation.

Twelve weeks later, participants returned to the laboratory for the third session, where a blood sample was taken to assess baseline antibody status and questionnaires were completed. Participants were then given a pneumococcal vaccination (Pneumovax II, Sanofi Pasteur MSD, UK, Batch no. NK527801616X) by a nurse. They remained in the laboratory for 15 minutes in case of adverse events (none occurred).

Four weeks after vaccination, participants returned to complete questionnaires, give a blood sample and have body composition, blood pressure, and aerobic fitness reassessed. Participants also wore a sealed pedometer for the week leading up to this session. Six months after vaccination, participants returned to the laboratory to complete questionnaires and give a blood sample. Figure 1 shows participant retention throughout the study.





**Figure 1:** CONSORT Flow Diagram.

### **Lifestyle physical activity interventions**

Participants received one of two interventions. Those in the lifestyle intervention group received a physical activity consultation by a trained practitioner, were given a pedometer (NL 1000, Missouri, USA) to use for the study duration, and received weekly prompts by telephone, email or text throughout the 16 week intervention. The physical activity consultation consisted of a booklet which the participant filled out whilst receiving guidance from the practitioner. This booklet asked the participant about the pros and cons of physical activity, personal barriers, goal setting, social support and lapse prevention. This was based upon previous successful physical activity consultation interventions (Kirk et al, 2007; Baker et al, 2008). The weekly prompts asked how they were doing with their physical activity participation and acted as an unstructured reminder to the participant. The educational control group were given an advisory leaflet on incorporating physical activity into their lifestyle (Walking the way to health, Natural England). This was used as a plausible physical activity intervention, which, alone, is unlikely to increase physical activity levels significantly.

### **Body composition analysis, blood pressure, and fitness testing**

Participants had their body fat measured using a Bioelectrical Body Composition Analyzer (Quantum X, RJL Systems, Michigan, USA) while lying supine with the upper body at 30°. Two sites on the right hand and foot were exfoliated (NuPrep, Weaver and Co, Colorado, USA) and degreased (Mediswab, UHS, UK) before tab electrodes (Q-trace, Kendall, Massachusetts, USA) were applied. The two signal electrodes were placed on the base of the middle finger and on the base of the third toe and the two detecting electrodes were placed on the dorsal aspect of the wrist and the ventral surface of the ankle. After a ten minute rest,

three measurements were recorded and the results analysed using Cyprus Body Composition Analysis software. Height and weight were also taken to calculate body mass index (BMI). Waist-hip ratio was calculated by dividing the circumference around the navel by the circumference of the widest point of the hips.

Blood pressure was taken with the body lying supine at 30° using a brachial cuff (Omron Intellisense M7, UK). After a 10 minute rest period, four measurements were taken at 2 minute intervals, the first being discarded in order to minimise the effects of ‘white coat syndrome’. A sub-maximal treadmill test was used to predict maximal oxygen consumption (VO<sub>2</sub> max) as a measure of aerobic fitness. After a warm-up, participants self-selected a ‘moderate’ walking pace, and then began a four step incremental stage test, with each stage lasting 5 minutes. The treadmill gradient increased after each stage by either 2 or 3 degrees depending on participants own estimation of fitness. Carbon dioxide and oxygen percentage, gas volume and temperature were measured using Douglas bags collecting expired air during the last minute of each 5 minute stage in order to ascertain estimated VO<sub>2</sub> max (Egan, 1999).

## **Questionnaires**

### **Physical Activity Status**

The Neighbourhood Physical Activity Questionnaire (NPAQ) (Giles-Corti et al, 2006) assessed the frequency and duration of recreational and transport related walking within and outside the participants’ neighbourhood. It is a 14-item measure that uses prompted recall to estimate how many minutes were spent walking in the past 7 days in all locations. Items in the questionnaire were summed to gain a total self reported number of minutes walking in the

past 7 days. Giles-Corti et al (2006) reported the intra-class correlation of total walking as excellent (0.91).

A modified version of the International Physical Activity Questionnaire (IPAQ) (<http://www.ipaq.ki.se>) was used to assess self-reported physical activity within the last 7 days. Items assessing walking behaviour were omitted to avoid duplication with the NPAQ. The questionnaire, therefore, collects information on number of days and time spent during the previous week participating in moderate- and vigorous-intensity physical activity. Data from the questionnaire were used to calculate the total number of metabolic equivalent of the task (MET) minutes accumulated per week. The test-retest Spearman's reliability coefficient for the IPAQ short form in the United Kingdom is 0.69 (Craig et al, 2003).

### **Psychological Status**

The 4-item Perceived Stress Scale (Cohen et al, 1983) was used for the assessment of nonspecific, appraised stress during the last month. The questionnaire consisted of 4 items where participants rated each statement on a 5 point scale (0-4) where 0 indicated 'never' and 4 'very often'. Items 4, 5, 7 and 8 were positive statements and reverse scored. The coefficient alpha for the PSS has been previously reported as 0.85 (Cohen et al, 1983).

In addition, affect was measured using the Russell Circumplex Questionnaire (Russell, 1980). This questionnaire measures affect based on 14 unipolar items placed in an integrated two-dimensional space. The two main affect dimensions reflect degrees of pleasantness-unpleasantness and arousal. Participants rated each emotion on a 5 point scale (1-5) with 1 representing 'very slightly/not at all' and 5 as 'very much'. Each dimension (pleasant-activated, pleasant-inactivated, unpleasant-activated, unpleasant-inactivated) consisted of 7 emotions, which were summed and divided by 7 to create a mean score for that dimension.

The Dartmouth COOP General Health Questionnaire (Nelson et al, 1987) is a 9-item questionnaire assessing various factors related to quality of life on a 1-5 point scale. The average test-retest intra class correlation over 2 weeks has been reported as .67 (Nelson et al, 1990). Higher quality of life is indicated by lower scores.

### **Smoking Behaviour**

Smoking behaviour was assessed using a questionnaire adapted from the Whitehall II study (Marmot et al., 1991). Participants were asked, on average, how much they smoked (0, 1–5, 6–10, 11–20, 21+ cigarettes per day); due to the low incidence of smokers, smoking status was further categorised into a dichotomous ‘yes’ or ‘no’ variable.

### **Blood sampling and pneumococcal antibody analysis**

Blood samples were taken by venepuncture from the antecubital vein in the arm at the time of vaccination, and at 4 weeks and 6 months post vaccination. Serum was collected in a 10ml plain red vacutainer tube (Becton–Dickinson, UK), which was allowed to clot at room temperature for 1 hour and then centrifuged at 3000rpm for 5 minutes. The separated serum was frozen at –20 °C for later antibody titre determination.

Luminex technology was used to assess eleven pneumococcal (Pn) IgG antibody serotypes (1, 3, 5, 6b, 7f, 9v, 14, 18c, 19a, 19f, 23f) contained in the pneumococcal vaccine. These were chosen as they are the strains most likely to cause pneumococcal disease within Europe (Denham and Clarke, 2005; Sleeman et al., 2001). Further details of this assay are described elsewhere (Ferraro et al., 2008). In short, carboxyl microspheres (Bio-Rad Labs, UK) were conjugated to individual purified pneumococcal polysaccharides (LGC Prochem/ATCC, UK) via poly-L-lysine. Seven four-fold dilutions of reference serum 89SF (Food and Drug

Administration, USA), beginning at 1:20, were made with diluent buffer (PBS with 0.05% Tween, 1% BSA, and 10.5µl of pneumococcus cell wall polysaccharide (Statens Serum Institute, Copenhagen, Denmark) for use as a standard curve. Serum samples were diluted 1:100 in diluent buffer that additionally contained 9.1µl purified pneumococcal serotype 22F (LCG, Reference Materials, Middlesex). 25µl of the conjugated microspheres (2500 per serotype) suspended in PBS-Tween were added to a filter membrane microtitre 96-well plate (Millipore Corp., Ireland) before washing and aspirating. Sera and standards (25µl per well) were transferred to the filter membrane microtitre plate and incubated with microspheres in the dark for 60 min at room temperature, with shaking. After incubation, washing and aspiration, 100µl of IgG-PE mouse anti-human secondary antibody (Southern Biotech, USA) diluted 1:200 with PBS Tween, was added to the wells. This was allowed to incubate for another 30 min in the dark, with shaking. Contents were then washed, aspirated and resuspended in 125µl of wash buffer and read on a Luminex 200 machine (Luminex Corp, TX, USA) programmed to collect a minimum of 100 microspheres per serotype. Acquisition software (BioPlex Software Manager, version 4, BioRad, Labs, CA, USA) was used to generate serotype antibody concentrations from a five parameter logistic curve fit. Serum Pn IgG levels are reported in µg/ml. Our data indicated that the intra and inter assay coefficient of variability were 8.0% and 10.4% respectively.

Serum samples reported as out of range values above or below the standard curve were re-run at a 1:500 dilution (n=31). Any remaining values which were out of range below the standard curve were assigned 0.01. If values were out of range above the curve, they were assigned the value of the top of the standard curve.

## Statistical Analysis

All analyses were carried out using SPSS statistical software version 19.0 (SPSS Inc, Chicago, IL). Differences between groups at baseline were analysed using one way analysis of variance (ANOVA) for interval data and Chi square for nominal variables. Repeated measures ANOVA with Group as a between subjects factor and Time (pre intervention, post intervention) as a within subjects factor was used to examine physiological, psychological and physical activity measures. As with previous research using self reported physical activity data (Rzewnicki et al, 2002), physical activity scores were skewed. Therefore, a log transformation ( $\log+1$ ) was used to approximate normal distribution (De Bourdeaudhuij and Sallis, 2002). For descriptive reporting, these values were exponentiated. Any physical activity scores which lay more than three standard deviations away from the mean were classed as outliers and excluded ( $n= 7$ ), which is reflected in the reduced degrees of freedom reported.

Due to the skewed distribution of the antibody data, titres were subject to  $\log^{10}$  transformation for each strain. Multivariate repeated measures ANOVA with antibody to each strain entered together as the dependent variables, Group as a between subjects factor and Time as a within subjects factor was used to assess change in IgG antibody titres. Due to the attrition rates between 4 weeks and 6 months, the multivariate repeated measures analyses were performed initially between baseline and 4 weeks post vaccination, and then with all three time points (baseline, 4 weeks and 6 months). Pairwise contrasts were used to investigate any differences between the three time points. Univariate results were then used to investigate any strain-specific differences. Clinically effective vaccine response was defined by achieving an absolute level  $0.35\mu\text{l/L}$  for at least 8/11 pneumococcal strains, in accordance with current

recommendations (WHO, 2005). Differences between groups in number of responders were analysed using Chi square analysis.

Linear regression analyses were performed to investigate if any variables predicted follow up antibody levels. The geometric mean at follow up (4 weeks or 6 months post vaccination antibody titres) was entered as the dependent variable, with baseline geometric mean (pre vaccination antibody titres) and group as step one of the regression. This was followed by the independent variable (physiological, psychological, physical activity measure) at step two.



## RESULTS

### Participant characteristics

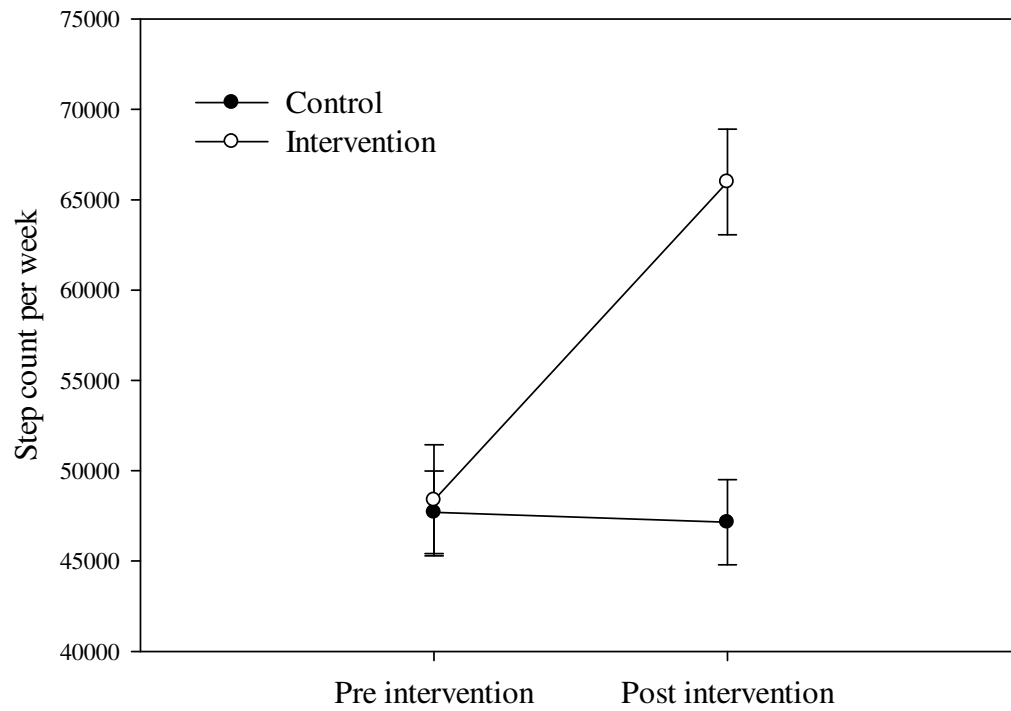
Table 2 compares the anthropometric, fitness, and psychological assessments for each group at baseline (pre intervention). Diastolic blood pressure showed a small, albeit significant, difference between groups ( $p = .04$ ). No other group differences were detected.

### Physical activity

Figure 2A displays the mean steps per week for each group before and after the 16 week study period. The results show a significant interaction effect, with the intervention group increasing one week step counts compared to the control group after 16 weeks ( $F_{(1, 67)} = 9.63$ ,  $p < .01$ ,  $\eta^2 = .13$ ). This assessment was confirmed by self reported walking behaviour, which likewise showed a significant Time  $\times$  Group interaction ( $F_{(1, 68)} = 11.25$ ,  $p < .01$ ,  $\eta^2 = .14$ ) (Figure 2B). There was a main effect of Time for the IPAQ, in which participants increased their weekly MET minutes of vigorous ( $F_{(1, 69)} = 7.17$ ,  $p < .01$ ,  $\eta^2 = .09$ ) and moderate ( $F_{(1, 64)} = 6.83$ ,  $p = .01$ ,  $\eta^2 = .10$ ) physical activity. However, neither vigorous ( $F_{(1, 69)} = 1.53$ ,  $p = .22$ ,  $\eta^2 = .02$ ) nor moderate ( $F_{(1, 64)} = 1.34$ ,  $p = .25$ ,  $\eta^2 = .02$ ) weekly physical activity showed a significant Time  $\times$  Group interaction.

	Intervention Group	Educational Control Group
BMI (kg/m <sup>2</sup> )	27.70 (5.29)	27.30 (4.32)
SBP	131.27 (14.61)	126.56 (16.76)
DBP	84.36 (9.19)*	80.18 (9.62)*
VO2.mls.kg	31.00 (6.73)	28.83 (7.70)
Body Fat %	39.53 (7.79)	39.29 (6.69)
Waist to Hip ratio	0.93 (0.05)	0.93 (0.05)
Baseline 7-day Step Count	47696 (15000)	48372 (19681)
Walking (min/week)	83.16 (90.97)	119.18 (103.53)
IPAQ Vigorous (MET mins/week)	40.06 (69.73)	80.19 (142.54)
IPAQ Moderate (MET mins/week)	127.05 (281.57)	244.33 (448.83)
PSS-4	5.25 (2.45)	5.30 (2.34)
Positive-Activated affect score	2.53 (0.61)	2.63 (0.54)
Positive-Inactivated affect score	3.29 (0.71)	3.24 (0.59)
Negative-Activated affect score	2.01 (0.68)	2.00 (0.66)
Negative-Inactivated affect score	2.09 (0.64)	2.10 (0.73)
Quality of life score	19.37 (3.22)	19.97 (4.32)

**Table 2. Comparison of mean (SD) anthropometric, fitness and psychological assessments at baseline.** Univariate ANOVA tests were performed to investigate differences between groups. \* denotes a significant difference between groups (p< .05).



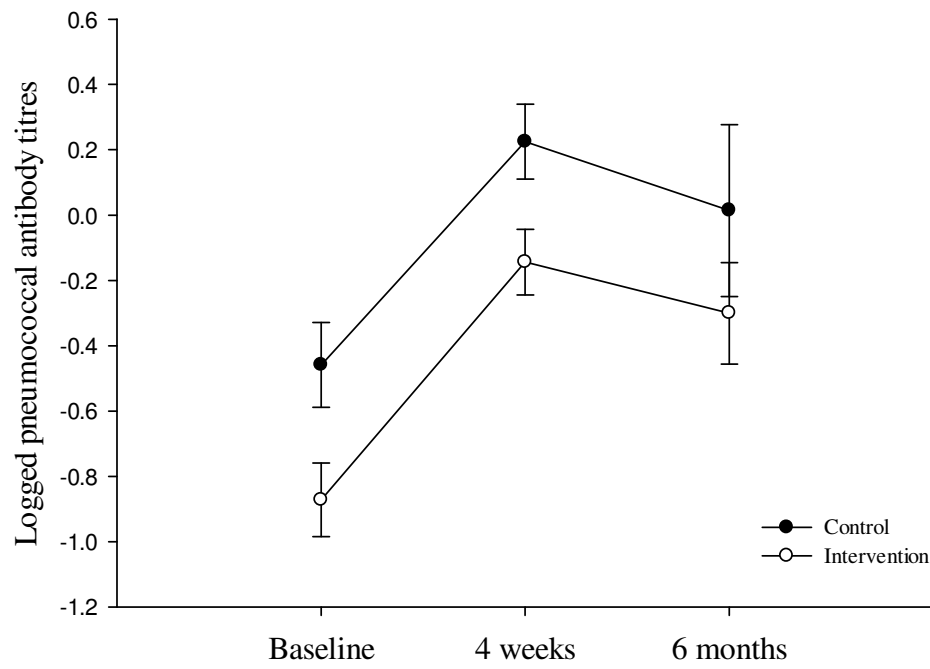
**Figure 2:** Mean (SE) one week step counts measured by pedometer before and after the 16 weeks in the intervention (open circles) and control (filled circles) groups.

## Antibody response to intervention

Figure 3 shows the average of the 11 logged pneumococcal strains between baseline, 4 week and 6 month follow up. A multivariate repeated measures ANOVA between baseline and 4 weeks, with the logged IgG titres to all 11 strains entered as dependent variables, revealed a significant overall increase in antibody titre ( $F_{(11, 61)} = 20.43, p < .001, \eta^2 = .79$ ), but no significant Time  $\times$  Group interaction ( $F_{(11, 61)} = 0.90, p = 0.55, \eta^2 = .14$ ). When examining the univariate analyses from the multivariate ANOVA, all 11 strains displayed a significant effect of Time ( $p$ 's all  $< .01$ ), apart from Pn19f ( $p = .10$ ). None of the 11 strains showed a significant Time  $\times$  Group interaction ( $p$ 's all  $> .18$ ). However, there was a trend towards a between subjects effect of Group ( $F_{(11, 61)} = 1.79, p = .08, \eta^2 = .24$ ), where those in the control group had higher antibody titres overall, compared to the intervention group. When investigating vaccination responders at 4 weeks post vaccination, 19/44 and 22/45 participants had clinically protective antibody levels in the intervention and control group, respectively. There were no differences between groups in those who achieved over  $0.35\mu\text{l}$  in 8 out of 11 strains at 4 weeks post vaccination ( $p = .35$ ).

When examining antibody responses at 6 months post vaccination, multivariate repeated measures ANOVA with baseline, 4 weeks and 6 months time points, again revealed a significant main effect of Time ( $F_{(22, 13)} = 3.95, p < .001, \eta^2 = .87$ ), but no significant Time  $\times$  Group interaction ( $F_{(22, 13)} = 1.44, p = .25, \eta^2 = .71$ ). However, there was a significant between subjects effect of Group ( $F_{(11, 24)} = 3.66, p < .01, \eta^2 = .63$ ), where those in the control group had overall significantly higher antibody titres, compared to the intervention group. Pairwise contrasts were performed to investigate the differences for each pneumococcal strain between the three time points. All but three strains (pn19a, pn19f and pn23f) were significantly increased above baseline at 4 weeks ( $p$ 's  $< .001-.01$ ), and all but 5 strains (pn7f, pn9v, pn19a,

pn19f and pn23f) remained significantly above baseline at 6 months ( $p$ 's  $<.001-.04$ ). Although antibody titres against most vaccine strains did not decrease significantly from 4 weeks to 6 months, three strains (pn1, pn9v and pn19f) showed a borderline reduction ( $p$ 's  $=.05-.09$ ) over this time period. When examining the univariate analyses from the multivariate ANOVA, 3 of the 11 pneumococcal strains were significantly higher in the control group overall ( $p$ 's all  $<.05$ ). When examining the interactions, the control group showed a significantly greater pn3 response over time, compared to the intervention group ( $F_{(2, 68)} = 3.19, p = .05, \eta^2 = .09$ ) and the intervention group showed a trend toward achieving a greater pn9 response, compared to the control group ( $F_{(2, 68)} = 3.03, p = .06, \eta^2 = .08$ ). There were no other significant interactions ( $p$ 's all  $>.17$ ).



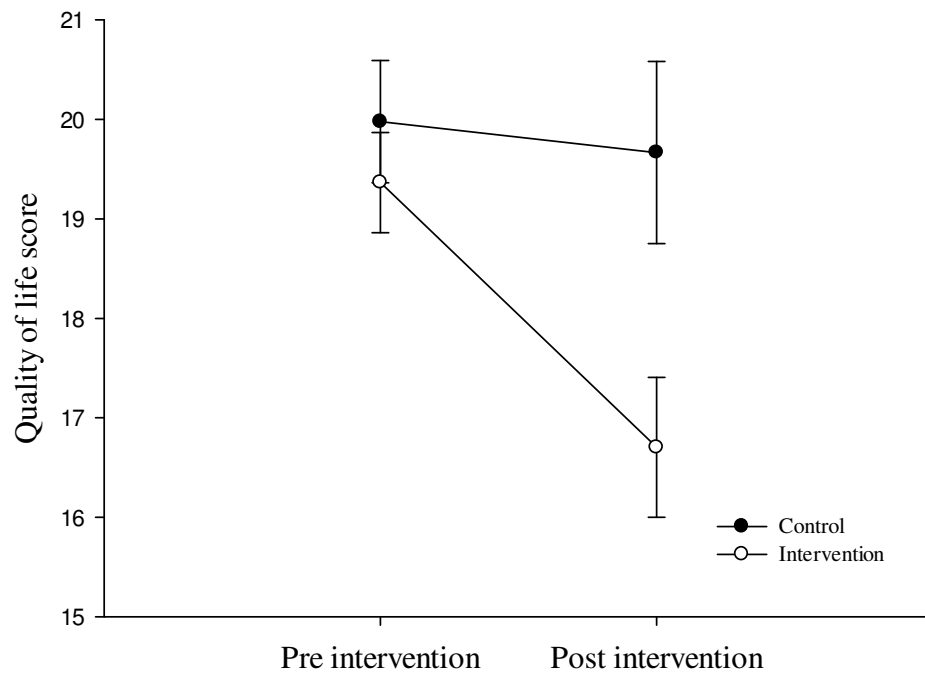
**Figure 3:** Mean (SE) of the 11 logged pneumococcal strains from pre vaccination to 4 weeks and 6 months post vaccination between intervention (open circles) and control (filled circles) groups.

### **Physiological responses to intervention**

The Time  $\times$  Group interaction for systolic blood pressure approached significance ( $F_{(1, 71)} = 3.22, p = .08, \eta^2 = .04$ ), whereby the intervention group had a greater decrease in SBP, compared to the control group. Overall, body mass index increased ( $F_{(1, 71)} = 4.37, p = .04, \eta^2 = .06$ ), which appeared driven by a significant increase in the control group ( $F_{(1, 34)} = 12.88, p < .01, \eta^2 = .28$ ), with no change in the intervention group ( $F_{(1, 37)} = 0.18, p = .67, \eta^2 = .01$ ); however, there was no significant Time  $\times$  Group interaction ( $F_{(1, 71)} = 1.88, p = .18, \eta^2 = .03$ ). No significant main or interaction effects were observed for diastolic blood pressure,  $VO_2$  max, waist-hip ratio or body fat percentage ( $p$ 's all  $>.16$ ).

### **Psychological responses to intervention**

Quality of life improved over the course of the intervention for both groups ( $F_{(1, 66)} = 7.89, p < .01, \eta^2 = .11$ ) and results showed a significant interaction whereby the intervention group showed a significant increase in their quality of life scores ( $F_{(1, 66)} = 4.44, p = .04, \eta^2 = .06$ ), compared to the control group (Figure 4). There was also a trend towards an overall significant decrease in the negative low activation dimension (e.g. depressed, droopy) of the Russell Circumplex model ( $F_{(1, 68)} = 3.77, p = .06, \eta^2 = .05$ ), but there was no significant interaction ( $F_{(1, 68)} = 1.71, p = .20, \eta^2 = .02$ ). Perceived stress and the three other dimensions of the Russell Circumplex model of affect showed no significant main or interaction effects ( $p$ 's all  $>.15$ ).



**Figure 4:** Mean (SE) quality of life scores over the 16 weeks between intervention (open circles) and control (filled circles) groups. Lower scores indicate a better quality of life score.



### **Predictors of vaccination response**

Linear regression, controlling for group, found that follow-up self-reported moderate MET minutes of physical activity per week significantly predicted antibody response at 4 weeks post vaccination,  $\beta = .23$ ,  $t = 2.53$ ,  $p = .01$ ,  $\Delta R^2 = .051$ , with those who reported more moderate MET minutes of physical activity having a higher antibody response. However, there was also an unexpected negative relationship between post intervention pedometer step counts and 4 week antibody response,  $\beta = -.21$ ,  $t = -2.03$ ,  $p = .05$ ,  $\Delta R^2 = .033$ . Finally, higher minutes of walking per week positively predicted antibody response at 6 months post vaccination,  $\beta = .24$ ,  $t = 2.02$ ,  $p = .05$ ,  $\Delta R^2 = .058$ . No other psychological, physiological or health behaviour measures predicted antibody response at 4 weeks or 6 months post vaccination ( $p$ 's all  $>.14$ ).

## **DISCUSSION**

This study examined whether a 16 week lifestyle physical activity intervention would improve antibody response to pneumococcal vaccination in women. Results revealed that the intervention successfully increased objective and subjective walking behaviour. There were no changes in any body composition or fitness measure, although the intervention group had a significant increase in quality of life post intervention, compared to the control group. Antibody levels showed the expected increase from baseline at 4 weeks and 6 months post-vaccination, but the intervention did not affect the magnitude of these responses at any time point. However, when controlling for group, it was observed that higher levels of moderate physical activity and walking behaviour were associated with a greater antibody response at 4 weeks and 6 months, respectively.

The present results did not corroborate previous research that found an increase in antibody response following a structured exercise intervention (Grant et al, 2008; Kohut et al, 2004; Woods et al, 2009). However, at 4 weeks and 6 months post vaccination, antibody response was predicted by measures of self-reported physical activity, controlling for group allocation. A possible explanation for this paradox is that habitual physical activity may be associated with other measures of a healthy lifestyle, which were not changed by our exercise intervention. For example, supplementary analyses revealed that higher levels of walking behaviour were significantly associated with lower BMI and body fat percentage both pre- and post-intervention (data not shown). It is plausible, therefore, that exercise is only beneficial for the antibody response to vaccination where it is associated with physiological changes, such as reduced adiposity and potential concomitant reductions in adipokines, such as IL-6 and tumour necrosis factor (Mujumdar et al, 2011; Ben Ounis et al, 2009). However, as BMI and body fat percentage were not independent predictors of the antibody response in

this dataset, this remains speculative. Future studies should consider using more detailed measures of adiposity, fat distribution, and adipokines to explore this issue.

Although, contrary to expectation, the intervention was not associated with changes in physiological measures, this is not without precedent. The "Walking for Wellbeing in the West" study found no significant changes in body fat percentage, body mass index or waist to hip ratio, despite an increase in walking behaviour (Baker et al, 2008). However, Nemoto et al (2007) found that high intensity interval walking training resulted in greater improvements in  $VO_2$  peak and SBP, compared to moderate intensity interval walkers, it is possible that this reflects a lack of sufficient walking intensity. Our participants may have increased their amount of walking per week, but neglected to maintain a moderate intensity throughout the walking period. However, as the current study did not measure exercise intensity or overall adherence to the intervention, it is difficult to test this hypothesis. Furthermore, in comparison to the previous exercise and vaccination literature (Kohut et al, 2004; Woods et al, 2009; Grant et al, 2008), the duration of our intervention was relatively short, which may not have been long enough to induce physiological or immunological benefits. A review examining the effect of a walking intervention on fitness, body composition and blood pressure concluded that a walking programme can induce significant beneficial changes to these physiological measurements (Murphy et al, 2007). However, these studies were all supervised, participants achieved an average of 70.1% of their predicted heart rate maximum during exercise, and study duration was an average of 34.9 weeks. Moreover, as shorter pedometer based interventions tend to result in a modest amount of weight loss (Richardson et al, 2008), a longer and more intense intervention may be required to induce significant physiological changes which, therefore, may be associated with an improved antibody response.

There may be other explanations as to why our intervention increased physical activity levels but failed to affect antibody response. Our middle aged participants were unlikely to be suffering from the age associated consequences of immunosenescence (Goodwin et al, 2006), whereas all previous structured intervention studies (Kohut et al, 2004; Grant et al, 2008, Woods et al 2009) used an elderly population. Moderate physical activity may not further enhance already robust immune responses. Such a pattern has been observed in the acute exercise and vaccination literature. For example, young healthy adults who completed a resistance exercise prior to vaccination showed a significant increase in antibody response, but only when the control group exhibited a poor response to vaccination (Edwards et al, 2007; 2008). Therefore, a physical activity intervention may only benefit those whose immune system is compromised. However, recent research has found that some reduction in antibody response is seen in adults 50-64 years old, who are not yet suffering from immunosenescence (Long et al, in press). Therefore, it is possible that our population, who are on average around 10 years younger, may also show a slight impairment in antibody response, compared to younger adults. Future research should investigate whether this type of lifestyle intervention can affect vaccination response in an older population.

Another factor which may explain our results is the type of vaccination. Our study used a pneumococcal vaccine which elicits a T cell independent antibody response, whereas the majority of previous physical activity intervention studies have used an influenza vaccination, which elicits a T cell dependent response. However, most longitudinal training studies have failed to show any changes in B cell proliferation (Walsh et al, 2011). If the primary mechanisms of exercise induced improvements in immune function is augmentation of T cell activity, then this may explain why no improvement was seen in a thymus independent vaccination response. Despite this, psychosocial factors have been shown to influence

antibody responses to both T cell dependent and independent vaccinations (Phillips et al, 2005), which suggests that both may be susceptible to neuroendocrine influence. Further research should address this issue systematically by directly comparing the responses of thymus dependent and independent antibody responses to exercise interventions.

In conclusion, a lifestyle physical activity intervention does not improve antibody response to a pneumococcal vaccination. This may be due to the intensity and/or duration of the intervention, the relatively young population, or the use of a T independent vaccination. However, this study raises other important implications for public health promotion. Worldwide, walking is promoted as an effective form of activity for a healthy lifestyle (Haskell et al, 2007; Levine, 2007). This study provides further evidence that lifestyle physical activity interventions can change walking behaviour, and are associated with improved quality of life. However, our findings also suggest that these changes may be insufficient to achieve significant physiological adaptations and, therefore, enjoy the putative physical health benefits.

## REFERENCES

- Abbas, A.K., Lichtman, A.H., 2006. *Basic Immunology; Functions and Disorders of the Immune System*. Elsevier, Philadelphia. 2nd edition.
- Azevedo, M.R., Araújo, C.L.P., Reichert, F.F., Siqueira, F.V., da Silva, M.C., Hallal, P.C., 2007. Gender differences in leisure-time physical activity. *Int J of Public Health* 52, 8-15.
- Baker, G., Gray, S.R., Wright, A., Fitzsimons, C., Nimmo, M., Lowry, R., Mutrie, N., and the Scottish Physical Activity Research Collaboration (SPARColl) 2008. The effect of a pedometer-based community walking intervention "Walking for Wellbeing in the West" on physical activity levels and health outcomes: a 12-week randomized controlled trial. *Int J of Behav Nutr Phys Act* 5, 44.
- Ben Ounis, O., Elloumi, M., Lac, G., Makni, E., Van Praagh, E., Zouhal, H., Tabka, Z., Amri, M., 2009. Two-month effects of individualized exercise training with or without caloric restriction on plasma adipocytokine levels in obese female adolescents. *Ann Endocrinol (Paris)* 70, 235-41.
- Bocher, W.O., Herzog-Hauff, S., Schlaak, J., Meyer zum Buschenfeld, K.H., Lohr, H.F., 1999. Kinetics of hepatitis B surface antigen-specific immune responses in acute and chronic hepatitis B or after HBs vaccination: Stimulation of the in vitro antibody response by interferon gamma. *Hepatology* 29, 238-44.
- Bravata, D.M., Smith-Spangler, C., Sundaram, V., Gienger, A.L., Lin, N., Lewis, R., Stave, C.D., Olkin, I., Sirard, J.R., 2007. Using pedometers to increase physical activity and improve health a systematic review. *JAMA* 298, 2296-2304.
- Burns, V.E., Carroll, D., Ring, C., Drayson, M., 2003. Antibody response to vaccination and psychosocial stress in humans: Relationships and mechanisms. *Vaccine* 21, 2523-2534.
- Burns, V.E., Gallagher, S., 2010. Antibody response to vaccination as a marker of in vivo immune function in psychophysiological research. *Neurosci Biobehav Rev* 35, 122-126.
- Cohen, S., Kamarck, T., Mermelstein, R., 1983. A global measure of perceived stress. *J Health Soc Behav* 24, 385-396.
- Craig, C.L., Marshall, A.L., Sjostrom, M., Bauman, A.E., Booth, M.L., Ainsworth, B.E., Pratt, M., Ekelund, U., Yngve, A., Sallis, J.F. and Oja, P., 2003. International physical activity questionnaire: 12-Country reliability and validity. *Med Sci Sports Exerc* 35, 1381-1395.

- De Bourdeaudhuij, I., Sallis, J., 2002. Relative contribution of psychosocial variables to the explanation of physical activity in three population-based adult samples. *Prev Med* 34, 279-288.
- Dunn, A.L., Marcus, B.H., Kampert, J.B., Garcia, M.E., Kohl, H.W., Blair, S.N., 1999. Comparison of lifestyle and structured interventions to increase physical activity and cardiorespiratory fitness. *JAMA* 281, 327-334.
- Edwards, K.M., Burns, V.E., Allen, L.M., McPhee, J.S., Bosch, J.A., Carroll, D., Drayson, M., Ring, C., 2007. Eccentric exercise as an adjuvant to influenza vaccination in humans. *Brain, Behav Immun* 21, 209-217.
- Edwards, K.M., Burns, V.E., Adkins, A.E., Carroll, D., Drayson, M., Ring, C., 2008. Meningococcal A vaccination response is enhanced by acute stress in men. *Psychosom Med* 70, 147-151.
- Egan, D., 1999. Analysis of expired air with gascalc: an automated spreadsheet. *Sport Science* 3, [sports.org/jour/9903/dje.html](http://sports.org/jour/9903/dje.html), (1549 words).
- Feldman, C., 1999. Pneumonia in the elderly. *Clin Chest Med* 20, 563–573.
- Ferraro, A.J., Drayson, M.T., Savage, C.O.S., MacLennan, I.C.M., 2008. Levels of autoantibodies, unlike antibodies to all extrinsic antigen groups, fall following B cell depletion with Rituximab. *Eur J Immun* 38, 292-298.
- Gallagher, S., Phillips, A.C., Ferraro, A.J., Drayson, M.T., Carroll, D., 2008. Psychosocial factors are associated with the antibody response to both thymus-dependent and thymus-independent vaccines. *Brain, Behav Immun* 22, 456-460.
- Giles-Corti, B., Timperio, A., Cutt, H., Pikora, T.J., Bull, F.C.L., Knuiman, M., Bulsara, M., Van Niel, K. and Shilton, T., 2006. Development of a reliable measure of walking within and outside the local neighborhood: RESIDE's neighborhood physical activity questionnaire. *Prev Med* 42, 455-459.
- Goodwin, K., Viboud, C., Simonsen, L., 2006. Antibody response to influenza vaccination in the elderly: a quantitative review. *Vaccine* 20, 1159-1169.
- 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, Behav Immun* 22, 923-932.
- Haskell, W.L., Lee, I.M., Pate, R.R., Powell, K.E., Blair, S.N., Franklin, B.A., Macera, C.A., Heath, G.W., Thompson, P.D., Bauman, A., 2007. Physical activity and public health: updated recommendation for adults from the American College of Sports Medicine and the American Heart Association. *Med Sci Sports Exerc* 39, 1423-1434.

- Heath, G.W., Ford, E.S., Craven, T.E., Macera, C.A., Jackson, K.L., Pate, R.R., 1991. Exercise and the incidence of upper respiratory tract infections. *Med Sci Sports Exerc* 23, 152-157.
- Kazdin, A.E. (1989) *Behaviour Modification in Applied Settings*, 4th edn. Brooks/Cole, Pacific Grove.
- Keylock, K.T., Lowder, T., Leifheit, K.A., Cook, M., Mariani, R.A., Ross, K., Kim, K., Chapman-Novakofski, K., McAuley, E., Woods, J.A., 2007. Higher antibody, but not cell-mediated, responses to vaccination in high physically fit elderly. *J Appl Physiol* 102, 1090-1098.
- Kirk, A., Mutrie, N., MacIntyre, P., Fisher, M., 2004. Promoting and maintaining physical activity in people with type 2 diabetes. *Am J Prev Med* 27, 289-296.
- Kirk, A.F., Barnett, J., Mutrie, N., 2007. Physical activity consultation for people with Type 2 diabetes: evidence and guidelines. *Diabet Med* 24, 809-816.
- Kohut, M.L., Arntson, B.A., Lee, W., Rozeboom, K., Yoon, K., Cunnick, J.E., McElhaney, J., 2004. Moderate exercise improves antibody response to influenza immunization in older adults. *Vaccine*, 22, 2298-2306.
- Kohut, M.L., Lee, W., Martin, A., Arnston, B., Russell, D.W., Ekkekakis, P., Yoon, K. J., Bishop, A., Cunnick, J. E., 2005. The exercise-induced enhancement of influenza immunity is mediated in part by improvements in psychosocial factors in older adults. *Brain, Behav Immun* 19, 357-366.
- Levine, J.A., 2007. Exercise: a walk in the park? *Mayo Clin Proc* 82, 797-798.
- Long, J.E., Bosch, J.A., Drayson, M.T., Ring, C., Campbell, J.P., Bhabra, J., Browne, D., Dawson, J., Harding, S., Lau, J., Burns, V.E., in press. *Brain Behav Immun*.
- Marmot, M.G., Davey Smith, G., Stansfield, S., Patel, C., North, F., Head, J., White, I., Brunner, E., Feeney, A., 1991. Health onequalities among British civil servants: the Whitehall II study. *Lancet* 337, 1387-1393.
- Marrie, T.J., 2000. Community-acquired pneumonia in the elderly. *Clin Infect Dis* 31, 1066-1078.
- 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 psychol* 20, 4-11.
- Marsland, A.L., Cohen, S., Rabin, B.S., Manuck, S.B., 2006. Trait positive affect and antibody response to hepatitis B vaccination. *Brain, Behav Immun* 20, 261-269.



- Martinez-Gonzalez, M.A., Varo, J.J., Sanots, J.L., De Irala, J., Gibney, M., Kearney, J., Martinez, J.A., 2001. Prevalence of physical activity during leisure time in the European Union. *Med Sci Sport Exerc* 33, 1142-1146.
- Mujumdar, P.P., Duerksen-Hughes, P.J., Firek, A.F., Hessinger, D.A., 2011. Long-term, progressive, aerobic training increases adiponectin in middle-aged, overweight, untrained males and females. *Scand J Clin Lab Invest* 71, 101-107.
- Murphy, M.H., Nevill, A.M., Murtagh, E.M., Holder, R.L., 2007. The effect of walking on fitness, fatness and resting blood pressure: a meta-analysis of randomised, controlled trial. *Prev Med* 44, 377-385.
- Nelson, E.C., Landgraf, J.M., Hays, R.D., Wasson, J.H., Kirk, J.W., 1990. The functional status of patients: How can it be measured in physicians' offices? *Med Care* 28, 1111-1126.
- Nelson, E.C., Wasson, J., Kirk, J., Keller, A., Clark, D., Dietrich, A., Stewart, A., Zubkoff, M., 1987. Assessment of function in routine clinical practice: description of the COOP chart method and preliminary findings. *J Chron Dis* 40, 55S-63S.
- Nemoto, K., Gen-no, H., Masuki, S., Okazaki, K., Nose, H., 2007. Effects of high-intensity interval walking training on physical fitness and blood pressure in middle-aged and older people. *Mayo Clin Proc* 82, 803-811.
- Nieman, D.C., 1993. Exercise and upper respiratory tract infection. *Res Sports Med* 4, 1-14.
- Nieman, D.C., 1994. Exercise, upper respiratory tract infection, and the immune system. *Med Sci Sport Exerc* 26, 128-139.
- 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, Behav Immun* 19, 325-333.
- Prochaska, J.A., Diclemente, C.C., 1986. Toward a comprehensive model of change, in: Miller, W.R., Heather, N. (Eds) *Treating Addictive Behaviors: processes of change*, pp. 3-27 (New York, Plenum).
- Richardson, C.R., Newton, T.L., Abraham, J.J., Sen, A., Jimbo, M., Swartz, A. M., 2008. A meta-analysis of pedometer-based walking interventions and weight loss. *Ann Fam Med* 6, 69-77.
- Russell, J.A., 1980. A circumplex model of affect. *J of Pers Soc Psychol* 39, 1161-1178.
- Rzewnicki, R., Auweele, Y.V., De Bourdeaudhuij, I., 2002. Addressing overreporting on the international physical activity questionnaire (IPAQ) telephone survey with a population sample. *Pub Health Nutr* 6, 299-305.

- Salmon, J., Owen, N., Crawford, D., Bauman, A., Sallis, J. F., 2003. Physical activity and sedentary behavior: A population-based study of barriers, enjoyment, and preference. *Health Psychol* 22, 178-188.
- Smith, T.P., Kennedy, S.L., Fleshner, M., 2004. Influence of age and physical activity on the primary in vivo antibody and T cell-mediated responses in men. *J Appl Physiol* 97, 491-498.
- 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. *Exerc Immun Rev* 17, 6-63.
- WHO., 2005. Recommendations for the production and control of pneumococcal conjugate vaccination.
- Woods, J.A., Keylock, K.T., Lowder, T., Vieira, V.J., Zelkovich, W., Dumich, S., Colantuano, K., Lyons, K., Leifheit, K., Cook, M., Chapman-Novakofski, K., McAuley, E. 2009. Cardiovascular exercise training extends influenza vaccine seroprotection in sedentary older adults: The immune function intervention trial. *J Am Geriatr Soc* 57, 2183-2191.

## CHAPTER FOUR

### DO PSYCHOSOCIAL FACTORS AND PHYSICAL ACTIVITY LEVELS PREDICT ANTIBODY RESPONSE TO VACCINATION IN HIV+ PATIENTS?

#### ABSTRACT

Individuals with higher psychological distress or lower activity levels have been reported to elicit poorer antibody responses to vaccination. As patients with HIV infection have already impaired immune function, and report higher levels of psychosocial distress and lower physical activity levels, it is possible that their antibody responses to vaccination are particularly susceptible to behavioural influence. This study investigates whether antibody responses to a bivalent glycoconjugate *Haemophilus influenzae* B and meningococcal C vaccine and a 23-valent pneumococcal polysaccharide vaccine are predicted by psychological factors and physical activity levels in an HIV+ patient cohort. One hundred and eighty three participants (age (SD) = 40.4 (9.5) yrs) (32.8% female) attended the HIV clinic on two separate occasions. At the first session, baseline antibody titres were determined and participants received either the *Haemophilus influenzae* B and meningococcal C vaccine or the 23-valent pneumococcal vaccine, or both, depending on whether they had previously received the vaccination. Participants also completed a questionnaire regarding their health behaviours, psychological status and physical activity levels. They then returned four weeks later for a follow up blood sample. Overall, although there was some indication that physical activity levels, social support and life events stress were associated with antibody response to pneumococcal strains, there was only limited evidence of psychosocial and behavioural effects on antibody response to vaccination.

## INTRODUCTION

In 2009, the number of people in the UK living with HIV reached an estimated 86,500, including 6630 newly diagnosed HIV infections (Presanis et al, 2010). The HIV virus causes a decline of CD4+ T cells (Klimas et al, 2008) and other immune changes, such as B cell hyperactivation and increases in plasma cytokines, which result in a proinflammatory state (Gleeson et al, 2011). These changes result in a poorer protection from opportunistic infections (Klimas et al, 2008), which further declines as the disease progresses (Wallace et al, 1993; Hirschtick et al, 1995). The speed at which CD4+ counts decline can be affected by various factors. For example, co-infections may increase, and antiretroviral therapy may decrease, the rate of disease progression (Palella et al, 2003; Lawn et al, 2001). Lifestyle factors, such as psychological stress and physical activity levels, have also shown some associations with HIV progression markers such as CD4+ counts and viral load (Leserman, 2008; Bopp et al, 2004; deSouza et al, 2008). However, the associations between these lifestyle factors and defence against opportunistic infection in HIV infected populations have received less attention. One model which has been used to investigate such relationships is the vaccine model (Burns and Gallagher, 2010; Phillips, 2011). This can be used as a general indication of immune function, as it is possible to measure the response to a standard dose of antigen and can test both T cell dependent and independent immune responses. In addition, the antibody response following vaccination can be used as a marker of vaccination efficacy, which is important for this population, due to their immunosuppression and increased susceptibility to infection (Wallace et al, 1993). The vaccination of HIV patients is also part of the UK national standard of care (BHIVA, 2008). Therefore, this would be an appropriate model to use in an HIV infected population.

Possible negative effects of psychological factors on immune function may be particularly relevant for the HIV infected population, as they tend to report higher levels of psychological distress, such as perceived stress and depression, compared to HIV– adults (Bing et al, 2001; Leserman, 2008). A number of studies have examined the impact of psychosocial factors upon markers of HIV progression, such as CD4+ count and viral load. For example, cross-sectional research suggests that people with lower CD4+ counts have a higher incidence of depression (Schroeksadel et al, 2008; Kaharuza et al, 2006). Furthermore, Ickovics et al (2001) found that chronically depressed women with CD4+ counts <200µl/ml had a higher mortality rate, compared to those with limited or no depressive symptoms. However, other longitudinal studies have shown that HIV progression and AIDS-related mortality have associations with both depression (Ickovics et al, 2001; Evans et al, 1997; Farinpour et al, 2003; Ironson et al, 2005; Leserman et al, 1999; 2003; 2007) and other psychological factors, such as trauma (Leserman et al, 1999; 2003), bereavement (Kemeny and Dean, 1995), stressful life events (Ironson et al, 2005; Evans et al, 1997) and perceived stress (Greeson et al, 2008; Remor et al, 2007). Overall, higher psychosocial distress in the HIV infected population tends to be related to lower CD4+ counts and a higher viral load.

The impact of psychological factors on other markers of immune function, such as antibody response to vaccination, has not been examined within the HIV infected population. However, in the HIV uninfected population, higher levels of psychosocial distress have been shown to affect the antibody response to vaccination, as a marker of immune function (Burns and Gallagher, 2010; Phillips, 2011). For example, higher numbers of stressful life events were associated with a reduced antibody response following hepatitis B vaccination in healthy medical students (Burns et al, 2002) and in chronically stressed populations, such caregivers, a reduced response to pneumococcal and influenza vaccination has been reported (Gallagher

et al, 2009; Glaser et al, 2000). It is particularly pertinent to investigate whether HIV infected patients' antibody responses are affected by higher levels of psychological distress because vaccinations tend to be less effective within this population and those with HIV experience a high mortality from opportunistic infections.

In addition to higher levels of psychological morbidity, the HIV infected population are also more likely to report lower levels of physical activity (Clingerman et al, 2003; Fillipas et al, 2008), compared to those without HIV infection. Some evidence suggests that mild to moderate regular physical activity can help HIV patients maintain positive wellbeing (Morgan and Goldston, 1997), decrease fatigue and increase muscle strength (Hand et al, 2009), and slow muscle wasting (Mustafa et al, 1999). However, the relationship between physical activity and HIV progression is less clear. Intervention studies conducted before the development of antiretroviral therapy (ART) tended to find that physical activity improved CD4+ counts and decreased viral load (Pothoff et al, 1994; LaPerriere et al, 1991; Perna et al, 1999). For example, Perna et al (1999) found that 45 minutes of cycling exercise, three times per week for 12 weeks, increased CD4+ counts in men and women who were compliant to the intervention. During the post-ART era, Bopp et al (2004) found that higher objective measures of physical activity were related to a lower viral load. Furthermore, deSouza et al (2008) found significant increases in CD4+ counts in elderly Brazilians following a resistance exercise programme. However, many cross sectional and longitudinal training studies, particularly those conducted post-ART, have failed to find a relationship between physical activity and HIV status (Smit et al, 2006; Clingerman et al, 2003; Ramírez-Marrero et al, 2004; Stringer et al, 1998; Smith et al, 2001; Baigis et al, 2002). It is possible that physical activity has less effect upon CD4+ count once patients are taking ART and, therefore, maintain relatively high and stable CD4+ counts. Additionally, these studies typically had

small participant numbers, high dropout rates and different approaches to the physical activity interventions, which may have also affected the results. Although it remains unclear whether physical activity levels are related to HIV progression, it is generally regarded as safe and is usually recommended by clinicians for HIV patients (O'Brien et al, 2010).

Moderate physical activity has also been shown to improve some aspects of immune function within a healthy population. For example, elderly participants who have a higher cardiovascular fitness show greater antibody responses to vaccination, compared to those who are less physically fit (Keylock et al, 2007; Smith et al, 2004). Furthermore, longitudinal training studies where participants carried out a moderate physical activity intervention have also shown significant improvements in antibody response to vaccination, compared to an attention-control group (Kohut et al, 2004; Grant et al, 2008; Woods et al, 2009). Therefore, as the physically active HIV- population tend to elicit an improved antibody response following vaccination, the same relationship may also be present within the HIV infected population. Furthermore, the relationships between psychological distress and physical activity often appear in the over 65 year old population, who tend to suffer from a reduced antibody response as a result of immunosenescence, making them a comparable population to HIV patients. Therefore, it is possible that similar results would be seen in those with HIV.

Our study examines whether antibody responses to a haemophilus influenzae B (HIB) and meningococcal C bivalent glycoconjugate vaccine and 23-valent pneumococcal polysaccharide vaccine are predicted by a range of psychological variables, such as perceived stress, depression and life events stress, and health behaviours, such as physical activity in an HIV patient cohort. It is hypothesised that HIV patients who report higher levels of psychosocial distress and lower levels of physical activity will have reduced antibody responses to the vaccinations at 4 weeks post vaccination.

## **METHODS**

### **Participants**

One hundred and eighty three participants from University Hospital Birmingham HIV outpatient clinic were recruited by study nurses or doctors. Prospective participants were screened for eligibility, which included HIV diagnosis, 18+ years old, not pregnant, currently attending the Selly Oak HIV clinic, and a good understanding of spoken and written English. This study was given ethical approval by the North Staffordshire Local Research Ethics Committee and was a sub-study from a larger study which investigated antibody responses to immunisations in HIV infected patients, compared to an HIV uninfected population. This sub-study deals specifically with the psychological and behavioural factors on the response to vaccination in the context of HIV infection.

### **Procedure**

During a routine appointment, patients were approached by their doctor or study nurse and informed about the study. Upon giving consent, participants filled out a questionnaire regarding previous vaccinations and illnesses. A blood sample was taken to measure baseline antibody titres and CD4+ count before participants were administered any vaccinations which they previously had not received. They were also given a pack of psychosocial and behavioural questionnaires to complete and return in their own time. Four weeks later, participants returned to the clinic to have a follow up blood sample taken.



## **Vaccinations**

Participants were only administered vaccinations which they had not previously received. Vaccinations were administered by a trained nurse and included 23-valent pneumococcal (Pneumovax II, Sanofi Pasteur) and bivalent glycoconjugate meningitis C and *Haemophilus influenzae* B (HIB; Mentorix, GlaxoSmithKline) vaccinations. The 23-valent pneumococcal polysaccharide vaccination (Pneumovax II, Sanofi Pasteur) contained 12 strains (pn1, pn3, pn4, pn5, pn6b, pn7f, pn9v, pn14, pn18c, pn19a, pn19f, pn23f). The bivalent glycoconjugate vaccination contained the HIB and meningococcal C strain.

## **Questionnaires**

### **Demographic questionnaires**

Participants completed a battery of questionnaires at baseline. In addition to the baseline questionnaire, participants were asked their employment status (professional, managerial/technical, skilled non-manual, skilled manual, partly skilled, unskilled, armed forces, unemployed), highest level of academic qualifications (postgraduate degree, undergraduate degree, A-levels or equivalent, GCSEs or equivalent, no academic qualifications) and the last time they missed taking their medications (within the past week, 1-2 weeks ago, 2-4 weeks ago, 1-3 months ago, more than 3 months ago, never skip medications). For analysis, employment status was further categorised into 'manual' (skilled manual, partly skilled, unskilled, armed forces), 'non-manual' (professional, managerial/technical, skilled non-manual) and 'unemployed'; academic status was categorised to 'up to college level', 'up to university level' and 'no academic qualifications';

and adherence was categorised to ‘missed medications within the last three months’, ‘missed medications more than three months ago’ and ‘never missed medications’.

### **Health Behaviours Questionnaire**

Health behaviours (smoking, alcohol consumption) were assessed using a questionnaire adapted from the Whitehall II study (Marmot et al., 1991). In this questionnaire, participants were asked, on average, how much they smoked (0, 1–5, 6–10, 11–20, 21+ cigarettes per day) and how much alcohol they drank (0, 1–5, 6–10, 11–20, 21–40, 40+ units per week). Smoking and drinking status were further categorised into a dichotomous ‘yes’ or ‘no’ variable.

### **Physical Activity Status**

The International Physical Activity Questionnaire (Craig et al, 2003) was used to assess self-reported physical activity within the last 7 days. The questionnaire collects information on number of days and time spent during the previous week participating in vigorous-intensity physical activity, moderate-intensity physical activity, walking behaviour and hours spent sitting on a weekday. Data from the questionnaire were calculated to find the total number of metabolic equivalent (MET) minutes spent carrying out activity at each of these intensities. The test-retest Spearman’s reliability coefficient for the IPAQ short form in the United Kingdom is 0.69 (Craig et al, 2003).

The Life Events Scale (Carroll et al, 2005; Ford et al, 1994) is a 48-item questionnaire which lists significant stressful life events. These were categorised under the following headings: ‘serious romantic relationships’, ‘relationships’, ‘deaths’, ‘work’, ‘housing’, ‘finances’, ‘general’ and ‘other’ events. The participant indicated ‘yes’ or ‘no’ to each stressor, and the cumulative number of stressors were totalled.

The 10-item Centre of Epidemiological Studies Depression Scale (CES-D) was used to measure depressive symptoms (Kohout et al, 1993) over the past 7 days. Participants indicated how often they experienced each item on a 4 point scale (Less than once a day, One-two days, Three-four days, Five-seven days per week). Items 5 and 8 were positive statements and reverse scored, and a score of 10 or more indicated possible clinical depression. It has been reported that the 10-item CES-D has a high test-retest reliability (Pearson's  $r = 0.83$ ) (Irwin et al, 1999).

Six items of the ENRICH Social Support Instrument (ESSI) were used to evaluate level of social support (Vaglio et al, 2004). The original seventh item was omitted as this item is not used in calculating the overall social support score. Participants rated their level of social support on a 5 point scale (None of the time, A little of the time, Some of the time, Most of the time, All of the time). The intra-class correlation coefficient was previously reported as 0.94, reflecting excellent reproducibility (Vaglio et al, 2004).

The Dartmouth COOP General Health Questionnaire (Nelson et al, 1987) is a 9-item questionnaire assessing various factors related to quality of life on a 1-5 point scale. The average test-retest intra class correlation over 2 weeks for six questions has been reported as .67 (Nelson et al, 1990). Higher quality of life is indicated by lower scores.

### **Antibody Analysis**

Luminex technology was used to assess antibody titres against 12 of the 23 pneumococcal (Pn) IgG antibody serotypes (types 1, 3, 5, 6b, 7f, 9v, 14, 18c, 19a, 19f, 23f), the meningococcal C strain, and HIB simultaneously (Whitelegg et al, in preparation). In short, carboxyl microspheres (Bio-Rad Labs, UK) were conjugated to individual purified

pneumococcal polysaccharides, meningococcal C or HIB (LGC Prochem/ATCC, UK) via poly-L-lysine. Seven four-fold dilutions of reference serum 89SF (Food and Drug Administration), beginning at 1:20, were made with diluent buffer (PBS with 0.05% Tween, 1% BSA, and 10.5µl of pneumococcus cell wall polysaccharide (Statens Serum Institute, Copenhagen, Denmark) for use as a standard curve. Serum samples were diluted 1:100 in diluent buffer that additionally contained 10µl purified pneumococcal serotype 22F (LCG, Reference Materials, Middlesex). 25µl of the conjugated microspheres (2500 per serotype) suspended in PBS-Tween were added to a filter membrane microtitre 96-well plate (Millipore Corp., Ireland) before washing and aspirating. Sera and standards (25µl per well) were transferred to the filter membrane microtitre plate and incubated with microspheres in the dark for 60 min at room temperature, with shaking. After incubation, washing and aspiration, 100µl of IgG-PE or IgM-PE mouse anti-human secondary antibody (Southern Biotech) diluted 1:200 with diluents buffer, was added to the wells. This was allowed to incubate for another 30 min in the dark, with shaking. Contents were then washed, aspirated and resuspended in 125µl of wash buffer and read on a Luminex 100 machine (Luminex Corp, TX, USA) programmed to collect a minimum of 50 microspheres per serotype. Acquisition software (BioPlex Software Manager version 4, BioRad, Labs, CA, USA) was used to generate serotype antibody concentrations from a five parameter logistic curve fit. Serum IgG levels are reported in µg/ml. Any antibody titres which were above or below the standard curve were assigned the top or bottom of the curve value.

## Statistical Analysis

Analyses were carried out using SPSS version 19.0 (SPSS Inc, Chicago, IL). Differences between CD4+ count groups at baseline were analysed using one way analysis of variance (ANOVA). Due to the skewed distribution of the antibody data, pneumococcal, meningococcal C and HIB titres were subject to  $\log^{10}$  transformation for each strain. Repeated measures ANOVA was used to report antibody response between baseline and 4 weeks post vaccination for the HIB, meningococcal C and 12 strains of the pneumococcal vaccine separately. Seroprotective status to the vaccination was also reported; seroprotective antibody status was defined as follow up titres  $>1\mu\text{l/mL}$  for HIB vaccine (WHO, 2006),  $>2\mu\text{l/mL}$  for the meningococcal C vaccine (Gold et al, 1979) and  $>0.35\mu\text{l/ml}$  for at least 8/12 strains of the pneumococcal vaccination (WHO, 2005).

Multiple linear regression analyses were used to investigate whether any psychological or physical activity variable significantly predicted follow up antibody response. Baseline antibody response was entered at step one of the regression. As previous research has shown that age can affect antibody response to vaccination (Grubeck-Loebenstein, 2010), this variable, and the two markers of HIV progression, CD4+ count and viral load, were also entered at step one of the regression. This was followed by the psychosocial (life events, social support, quality of life, depression) or physical activity (Vigorous MET minutes of physical activity, moderate MET minutes of physical activity, walking MET minutes of physical activity) variables at step two. This was repeated, in turn, for the HIB, meningococcal C, each of the 12 pneumococcal strains (pn1, pn3, pn4, pn5, pn6b, pn7f, pn9v, pn14, pn18c, pn19a, pn19f, pn23f) and a mean z score of the 12 pneumococcal strains, which were each entered as the dependent variable in turn.

## **RESULTS**

### **Participant characteristics**

Table 1 shows participant psychosocial and physical activity levels at baseline. The mean age of participants was 40.4 (SD±9.5) years and 32.8% were women. The majority of participants were either black (48.6%) or white (47.6%) with the remainder self reporting being of mixed race (2.7%) or ‘other’ ethnic origins (1.1%). At study entry, 76.5% of the participants were taking antiretroviral medication. Adherence to medication data showed that 62.5% of participants never missed medications, 13.1% missed medications more than 3 months ago, but 24.4% had missed medication in the last 3 months. The participants’ occupations were predominantly non manual (49.3%), whereas 20.1% were engaged in manual work and 30.6% were unemployed. Most were educated to at least college level (51.7%), with 37.6% of those reporting a university education, although 10.7% of the cohort had no academic qualifications. The prevalence of smoking was 28.7% and 71.3% reported drinking alcohol. The participants’ average CD4+ counts were 532.8/μL (SD±223.1) and the average viral load was 9946.9 copies/mL (SD±41,892.1).

### **Antibody responses to vaccination**

Table 2 shows the geometric means of the baseline and follow up antibody titres for the HIB, meningococcal C and pneumococcal strains. Seroprotective antibody titres were achieved at follow up by 75% of the participants for HIB (antibody titres greater than 1μl/mL (WHO, 2006)), 59.5% for meningococcal C (antibody titres above 2μl/mL (Gold et al, 1979)) and 72.2% for pneumococcal (antibody titres over 0.35μl/ml for at least 8/12 pneumococcal strains (WHO, 2005)).

Measures	
IPAQ Vigorous MET minutes/week	816 (1778)
IPAQ Moderate MET minutes/week	626 (1296)
IPAQ Walking MET minutes/week	1258 (1574)
Social support (ESSI)	19.2 (8.0)
Quality of life (Dartmouth COOP)	22.0 (7.8)
Stressful life events (LES)	8.7 (7.0)
Depression (CES-D)	11.6 (7.4)

**Table 1:** Mean and standard deviation of participant (n=183) psychological and physical activity characteristics.

	Baseline GM (95% CI)	Follow up (GM, 95% CI)	Degrees of freedom	F	<i>p</i>	$\eta^2$
HIB	0.82 (0.69-0.95)	1.99 (1.79-2.18)	(1, 129)	231.63	<.001	.64
Men C	0.49 (0.39-0.59)	1.73 (1.57-1.90)	(1, 127)	334.58	<.001	.73
Pn1	0.64 (0.56-0.72)	1.26 (1.12-1.39)	(1, 107)	104.62	<.001	.49
Pn3	0.69 (0.59-0.79)	0.94 (0.81-1.08)	(1, 109)	22.90	<.001	.17
Pn4	0.52 (0.44-0.60)	0.78 (0.67-0.89)	(1, 109)	47.02	<.001	.30
Pn5	0.66 (0.56-0.76)	1.33 (1.18-1.48)	(1, 109)	106.65	<.001	.50
Pn6b	0.82 (0.70-0.93)	1.21 (1.06-1.35)	(1, 109)	74.10	<.001	.41
Pn7f	0.89 (0.78-0.99)	1.46 (1.32-1.60)	(1, 109)	99.66	<.001	.46
Pn9v	0.73 (0.62-0.84)	1.31 (1.17-1.46)	(1, 109)	113.07	<.001	.51
Pn14	1.31 (1.14-1.48)	1.74 (1.56-1.93)	(1, 109)	54.40	<.001	.33
Pn18c	0.85 (0.73-0.97)	1.43 (1.27-1.60)	(1, 109)	104.08	<.001	.49
Pn19a	1.01 (0.88-1.14)	1.49 (1.33-1.64)	(1, 109)	89.00	<.001	.45
Pn19f	0.84 (0.73-0.94)	1.26 (1.12-1.40)	(1, 109)	62.67	<.001	.37
Pn23f	0.69 (0.61-0.78)	1.18 (1.04-1.32)	(1, 109)	94.92	<.001	.47
GM of all 12 Pn strains	0.68 (0.63-0.73)	1.11 (1.03-1.20)	(1, 109)	249.21	<.001	.70

**Table 2:** Geometric means (GM) and 95% confidence intervals (CI) of the baseline and follow up antibody titres for the HIB, meningococcal C and 12 pneumococcal strains with repeated measures ANOVA statistics.



## Predictors of antibody response to vaccination

Table 3 shows the multiple linear regression analyses, which control for baseline antibody titre, age, CD4+ count and viral load at step one. The psychological (social support, quality of life, life events, depression) and physical activity (vigorous MET minutes of physical activity, moderate MET minutes of physical activity, walking behaviour MET minutes) variables were then entered, in turn, at step two, and follow up antibody response was the dependent variable.

Higher numbers of life events were significantly related to a lower response to pn1 ( $\beta = -.17$ ,  $t = -2.03$ ,  $p = .05$ ,  $\Delta R^2 = .028$ ) and higher social support was significantly related to a higher antibody response to pn3 ( $\beta = .13$ ,  $t = 2.00$ ,  $p = .05$ ,  $\Delta R^2 = .017$ ). When examining the physical activity variables as predictors, higher vigorous MET minutes of physical activity significantly predicted higher antibody response to the pn1 ( $\beta = .28$ ,  $t = 3.37$ ,  $p < .01$ ,  $\Delta R^2 = .073$ ) and pn6b ( $\beta = .15$ ,  $t = 2.38$ ,  $p = .02$ ,  $\Delta R^2 = .021$ ) strains. Similarly, higher moderate MET minutes of physical activity predicted antibody response to the pn1 ( $\beta = .28$ ,  $t = 3.07$ ,  $p < .01$ ,  $\Delta R^2 = .073$ ), pn6b ( $\beta = .16$ ,  $t = 2.41$ ,  $p = .02$ ,  $\Delta R^2 = .023$ ) and pn18c ( $\beta = .17$ ,  $t = 2.33$ ,  $p = .02$ ,  $\Delta R^2 = .027$ ) strains. No other psychological or physical activity variable significantly predicted antibody response to the pneumococcal, meningococcal or HIB strains ( $p$ 's all  $> .05$ ).

	HIB	Men C	Pn z- scored mean	Pn1	Pn3	Pn4	Pn5	Pn6b	Pn7f	Pn9v	Pn14	Pn18c	Pn19a	Pn19f	Pn23f
Vigorous MET minutes per week	.13	-.02	.10	.28**	.08	-.06	.15	.15*	.06	.04	.08	.04	-.03	.02	.03
Moderate MET minutes/week	.08	.03	.10	.28**	.10	-.03	-.01	.16*	.08	-.02	.04	.17*	-.10	.04	.05
Walking MET minutes/week	.05	-.15	.04	.04	.02	.04	-.06	.05	.02	-.01	.01	.03	-.11	-.05	-.02
Social support	.00	.09	.01	.05	.13*	-.03	.03	-.01	.05	.02	-.02	.09	-.03	.07	.05
Life events	.02	-.01	-.03	-.17*	.01	-.02	-.08	-.02	-.06	-.09	.03	-.10	.02	-.06	-.03
Quality of life	.13	.11	.02	-.08	-.12	.04	-.01	-.07	-.04	.02	.02	-.05	.07	.02	-.00
Depression	-.00	.08	.03	-.05	-.10	.05	.02	-.06	-.08	.00	.03	-.06	.07	-.05	-.03

**Table 3:** Standardised beta coefficients for psychosocial and physical activity variables predicting antibody response. \*indicates  $p < .05$  \*\*indicates  $p < .01$ .

## DISCUSSION

This study examined the association between demographic, psychological, and physical activity variables and antibody responses to vaccination in HIV infected participants. The results found that HIB, meningococcal C and pneumococcal vaccinations induced seroprotective responses in 75%, 60% and 72% of the participants respectively. Higher vigorous and moderate physical activity levels predicted a greater antibody response to pneumococcal strains 1, 6b and 18c, but not to the meningococcal C and HIB vaccination. Furthermore, reporting more stressful life events predicted antibody response to the pn1 strain and higher social support predicted antibody response to the pn3 strain; there were no other significant predictors of any other vaccine strain.

Although these results suggest that physical activity and psychosocial variables are associated with some strains from the pneumococcal vaccination, on the whole, only a minority of relationships were significant. Therefore, these findings must be taken cautiously due to the large number of analyses carried out. Despite this, the few relationships which were found are in line with previous literature. For example, higher antibody responses to vaccination have been shown in older adults who are more physically active (Keylock et al, 2007; Smith et al, 2004; Kohut et al, 2004; Grant et al, 2008; Woods et al, 2009), and also in younger and older adults with higher levels of social support and life events (Burns et al, 2003; Phillips et al, 2006; Phillips, 2011). We also observed that the relationships between psychological and physical activity status with antibody response were specific to the pneumococcal strains, and such strain specificity has been found in a number of studies conducted in the healthy population (Gallagher et al, 2008; Phillips et al, 2005; Burns and Gallagher, 2010). One explanation for these results may be the variation in antibody response, psychosocial or

physical activity levels. However, the HIB and meningococcal C titres had a larger variation, compared to all but one (pn3) of the pneumococcal strains. Furthermore, psychosocial and physical activity data showed a larger variation within this study, compared to previous literature (Long et al, in press). This suggests that the largely null findings are unlikely to be due to low levels of variation in the data. It is possible that these relationships may have appeared due to the larger number of strains within the pneumococcal vaccine, or perhaps that specific T independent strains are more susceptible to psychosocial and physical activity influence.

There may be a number of reasons as to why this study did not find more significant relationships between psychosocial factors, physical activity and antibody response to vaccination in HIV+ patients. These results showed relatively low effect sizes, and a power analysis indicated that to detect significance of a small effect size (.02), a sample size of around 395 participants would be needed. Therefore, it is possible that a larger number of participants would be needed in this sample in order to find significant results. Another explanation for these results may be that the majority of patients were taking antiretroviral therapy (ART) and therefore had relatively high CD4+ counts. The literature prior to the advent of ART tended to find significant relationships between physical activity status and HIV progression (MacArthur et al, 1993; Pothoff et al, 1994; LaPerriere et al, 1991; Perna et al, 1999), whereas in studies where patients were taking ART, fewer significant relationships have been found (Bopp et al, 2004, deSouza et al, 2008). It may be that antibody responses in participants who have stable CD4+ counts and are relatively healthy are less affected by psychosocial factors or physical activity status.

The findings of the current study should be interpreted in light of a number of potential limitations. Although cross sectional research can suggest a relationship between lifestyle

factors and antibody response, it cannot imply causation or elucidate mechanisms, and it may be confounded by other unmeasured variables. An intervention trial, in which HIV+ patients were randomly allocated to an exercise intervention would help address these limitations. In addition, some studies have indicated that HIV patients overestimate their physical activity levels when using the IPAQ (Fillipas et al, 2010; Ramirez-Marrero et al, 2008). An objective measure, such as using an accelerometer, may be a more accurate method to measure physical activity in those with HIV, especially as studies using this methodology in the post ART era have found a significant relationship between this physical activity measure and HIV progression (Bopp et al, 2004; deSouza et al, 2008). Despite this, questionnaires can provide a measure of physical activity, and the IPAQ has shown reliability and validity in many countries (Craig et al, 2003). Finally, our study examined both a T independent pneumococcal vaccination and a meningococcal C and HIB bivalent glycoconjugate vaccination, which elicit a T dependent immune response despite being polysaccharide antigens. Although both types of antibody response could be affected by HIV infection (Kroon et al, 1994; Moir and Fauci, 2009), we did not examine antibody response to a purely protein antigen, such as influenza. Nonetheless, HIV patients often suffer from opportunistic bacterial infections, such as pneumonia and meningitis, and, therefore it is clinically important to examine the efficacy of both types of vaccination in the HIV infected population.

In summary, this study found that HIV patients' antibody responses to the pneumococcal, meningococcal and HIB vaccinations showed limited associations with psychological factors and physical activity behaviour. However, improving psychosocial status and physical activity levels can benefit those with HIV infection, such as by slowing muscle wasting, improving cardiovascular and lipid abnormalities, and improving quality of life (Macallan,

1999; O'Brien et al, 2010; Terry et al, 1999). Therefore, psychological and physical activity interventions in HIV+ participants remain important priorities for future research.

## REFERENCES

- Baigis, J., Korniewicz, D.M., Chase, G., Butz, A., Jacobson, D., Wu, A.W., 2002. Effectiveness of a home-based exercise intervention for HIV-infected adults: A randomized trial. *J Assoc Nurses AIDS Care* 13, 33-45.
- BHIVA., 2008. British HIV association guidelines for the treatment of HIV-1-infected adults with antiretroviral therapy 2008. *HIV Medicine* 9, 563-608.
- Bing, E.G., Burnam, M.A., Longshore, D., Fleishman, J.A., Sherbourne, C.D., London, A.S., Turner, B.J., Eggan, F., Beckman, R., Vitiello, B., Morton, S.C., Orlando, M., Bozzette, S.A., Ortiz-Barron, L., Shapiro, M., 2001. Psychiatric disorders and drug use among human immunodeficiency virus-infected adults in the United States. *Arch Gen Psychiatry* 58, 721-728.
- Bopp, C.M., Phillips, K.D., Fulk, L.J., Dudgeon, W.D., Sowell, R., Hand, G.A., 2004. Physical activity and immunity in HIV-infected individuals. *AIDS Care* 16, 387-393.
- Burns, V.E., Carroll, D., Ring, C., Harrison, L.K., Drayson, M., 2002. Stress, coping, and hepatitis B antibody status. *Psychosom Med* 64, 287-293.
- Burns, V.E., Carroll, D., Ring, C., Drayson, M., 2003. Antibody response to vaccination and psychosocial stress in humans: relationships and mechanisms. *Vaccine* 21, 2523-2534.
- Burns, V.E., Gallagher, S., 2010. Antibody response to vaccination as a marker of in vivo immune function in psychophysiological research. *Neurosci Biobehav Rev* 35, 122-126.
- Carroll, D., Phillips, A.C., Ring, C., Der, G., Hunt, K., 2005. Life events and hemodynamic stress reactivity in the middle-aged and elderly. *Psychophysiology* 42, 269-276.
- Clingerman, E.M., 2003. Participation in physical activity by persons living with HIV disease. *J Assoc Nurses AIDS Care* 14, 59-70.
- Craig, C.L., Marshall, A.L., Sjostrom, M., Bauman, A.E., Booth, M.L., Ainsworth, B.E., Pratt, M., Ekelund, U., Yngve, A., Sallis, J.F., Oja, P., 2003. International physical activity questionnaire: 12-country reliability and validity. *Med Sci Sports Exer* 35, 1381-1385.
- Evans, D.L., Leserman, J., Perkins, D.O., Stern, R.A., Murphy, C., Zheng, B., Gettes, D., Longmate, J.A., Silva, S.G., van der Horst, C.M., Hall, C.D., Folds, J.D., Golden, R.N., Petitto, J.M., 1997. Severe life stress as a predictor of early disease progression in HIV infection. *Am J Psychiatry* 154, 630-634.
- Farinpour, R., Miller, E.N., Satz, P., Selnes, O.A., Cohen, B.A., Becker, J.T., Skolasky, R.L., Visscher, B.R., 2003. Psychosocial risk factors of HIV morbidity and mortality: Findings from the Multicenter AIDS Cohort Study (MACS). *J Clin Exp Neuropsychol* 25, 654-670.

- Fillipas, S., Bowtell-Harris, C.A., Oldmeadow, L.B., Cicuttini, F., Holland, A.E., Cherry, C.L., 2008. Physical activity uptake in patients with HIV: who does how much? *Int J STD AIDS* 19, 514-518.
- Ford, G., Ecob, R., Hunt, K., Macintyre, S., West, P., 1994. Patterns of class inequality in health through the lifespan: class gradients at 15, 35 and 55 years in the west of Scotland. *Soc Sci Med* 39, 1037-1050.
- Gallagher, S., Phillips, A.C., Ferraro, A.J., Drayson, M.T., Carroll, D., 2008. Psychosocial factors are associated with the antibody response to both thymus-dependent and thymus-independent vaccines. *Brain Behav Immun* 22, 456-460.
- Gallagher, S., Phillips, A.C., Drayson, M.T., Carroll, D., 2009. Parental caregivers of children with developmental disabilities mount a poor antibody response to pneumococcal vaccination. *Brain Behav Immun* 23, 338-346.
- Glaser, R., Sheridan, J., Malarkey, W.B., MacCallum, R.C., Kiecolt-Glaser, J.K., 2000. Chronic stress modulates the immune response to a pneumococcal pneumonia vaccine. *Psychosom Med* 62, 804-807.
- Gleeson, M., Bishop, N.C., Stensel, D.J., Lindley, M.R., Mastana, S.S., Nimmo, M.A., 2011. The anti-inflammatory effects of exercise: mechanisms and implications for the prevention and treatment of disease. *Nat Rev Immunol* 11, 607-615.
- Gold, R., Lepow, M.L., Goldschneider, I., Draper, T.F., Gotschlich, E.C., 1979. Kinetics of antibody production to group A and group C meningococcal polysaccharide vaccines administered during the first six years of life: Prospects for routine immunization of infants and children. *J Infect Dis* 140, 690-698.
- 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 Behav Immun* 22, 923-932.
- Greeson, J.M., Hurwitz, B.E., Llabre, M.M., Schneiderman, N., Penedo, F.J., Klimas, N.G., 2008. Psychological distress, killer lymphocytes and disease severity in HIV/AIDS. *Brain Behav Immun* 22, 901-911.
- Grubeck, L., 2010. Fading immune protection in old age: Vaccination in the elderly. *J Comp Pathol* 142, Supplement 1, S116-S119.
- Hand, G.A., Lysterly, G.W., Jagers, J.R., Dudgeon, W.D., 2009. Impact of aerobic and resistance exercise on the health of HIV-infected persons. *Am J Lifestyle Med* 3, 489-499.



- Hirschtick, R.E., Glassroth, J., Jordan, M.C., Wilcosky, T.C., Wallace, J.M., Kvale, P.A., Markowitz, N., Rosen, M.J., Mangura, B.T., Hopewell, P.C., 1995. Bacterial pneumonia in persons infected with the human immunodeficiency virus. *N Engl J Med* 333, 845-851.
- Ickovics, J.R., Hamburger, M.E., Vlahov, D., Schoenbaum, E.E., Schuman, P., Boland, R.J., Moore, J., HIV Epidemiology Research Study Group, 2001. Mortality, CD4 cell count decline, and depressive symptoms among HIV-seropositive women: Longitudinal analysis from the HIV epidemiology research study. *JAMA* 285, 1466-1474.
- Ironson, G., O'Cleirigh, C., Fletcher, M.A., Laurenceau, J.P., Balbin, E., Klimas, N., Schneiderman, N., Solomon, G., 2005. Psychosocial factors predict CD4 and viral load change in men and women with human immunodeficiency virus in the era of highly active antiretroviral treatment. *Psychosom Med* 67, 1013-1021.
- Irwin, M., Artin, K.H., Oxman, M.N., 1999. Screening for depression in the older adult: Criterion validity of the 10-Item Center for Epidemiological Studies Depression Scale (CES-D). *Arch Intern Med* 159, 1701-1704.
- Kaharuza, F., Bunnell, R., Moss, S., Purcell, D., Bikaako-Kajura, W., Wamai, N., Downing, R., Solberg, P., Coutinho, A., Mermin, J., 2006. Depression and CD4 cell count among persons with HIV infection in Uganda. *AIDS Behav* 10, 105-111.
- Kemeny M.E., Dean, L., 1995. Effects of AIDS-related bereavement on HIV progression among New York City gay men. *AIDS Educ Prev* 7, 36-47.
- Keylock, K.T., Lowder, T., Leifheit, K.A., Cook, M., Mariani, R.A., Ross, K., Kim, K., Chapman-Novakofski, K., McAuley, E., Woods, J.A., 2007. Higher antibody, but not cell-mediated, responses to vaccination in high physically fit elderly. *J Appl Physiol* 102, 1090-1098.
- Klimas, N., Koneru, A.O., Fletcher, M.A., 2008. Overview of HIV. *Psychosom Med* 70, 523-530.
- Kohout, F.J., Berkman, L.F., Evans, D.A., Cornoni-Huntley, J., 1993. Two shorter forms of the CES-D (Center for Epidemiological Studies Depression) depression symptoms index. *J Aging Health* 5, 179-193.
- Kohut, M.L., Arntson, B.A., Lee, W., Rozeboom, K., Yoon, K.J., Cunnick, J.E., McElhaney, J., 2004. Moderate exercise improves antibody response to influenza immunization in older adults. *Vaccine* 22, 2298-2306.
- Kroon, F.P., van Dissel, J.T., de Jong, J.C., van Furth, R., 1994. Antibody response to influenza, tetanus and pneumococcal vaccines in HIV-seropositive individuals in relation to the number of CD4+ lymphocytes. *AIDS* 8, 469-76.
- LaPerriere, A., Fletcher, M.A., Antoni, M.H., Klimas, A., Ironson, G., Schneiderman, N., 1991. Aerobic exercise training in an AIDS risk group. *Int J Sports Med* 12, S53-S57.

- Lawn, S.D., Butera, S.T., Folks, T.M., 2001. Contribution of immune activation to the pathogenesis and transmission of human immunodeficiency virus type 1 infection. *Clin Microbiol Rev* 14, 753-777.
- Leserman, J., Jackson, E.D., Petitto, J.M., Golden, R.N., Silva, S.G., Perkins, D.O., Cai, J., Folds, J.D., Evans, D.L., 1999. Progression to AIDS: The effects of stress, depressive symptoms, and social support. *Psychosom Med* 61, 397-406.
- Leserman, J., 2003. HIV disease progression: depression, stress, and possible mechanisms. *Biol Psychiatry* 54, 295-306.
- Leserman, J., Pence, B.W., Whetten, K., Mugavero, M.J., Thielman, N.M., Swartz, M.S., Stangl, D., 2007. Relation of lifetime trauma and depressive symptoms to mortality in HIV. *Am J Psychiatry* 164, 1707-1713.
- Leserman, J., 2008. Role of depression, stress, and trauma in HIV disease progression. *Psychosom Med* 70, 539-545.
- Long, J.E., Bosch, J.A., Drayson, M.T., Ring, C., Campbell, J.P., Bhabra, J., Browne, D., Dawson, J., Harding, S., Lau, J., Burns, V.E., in submission. *Brain Behav Immun*.
- Macallan, D.C., 1999. Wasting in HIV infection and AIDS. *J Nutr* 129, 238S-242S.
- Marmot, M.G., Stansfeld, S., Patel, C., North, F., Head, J., White, I., Brunner, E., Feeney, A., Marmot, M.G., Smith, G.D., 1991. Health inequalities among British civil servants: the Whitehall II study. *Lancet* 337, 1387-1393.
- Moir, S., Fauci, A.S., 2009. B cells in HIV infection and disease. *Nat Rev Immunol* 9, 235-245.
- Morgan, W. P., Goldston, S. E., 1987. Exercise and mental health. Washington, DC, Hemisphere.
- Mustafa, T., Sy, F.S., Macera, C.A., Thompson, S.J., Jackson, K.L., Selassie, A., Dean, L.L., 1999. Association between exercise and HIV disease progression in a cohort of homosexual men. *Ann Epidemiol* 9, 127-131.
- Nelson, E.C., Wasson, J., Kirk, J., Keller, A., Clark, D., Dietrich, A., Stewart, A., Zubkoff, M., 1987. Assessment of function in routine clinical practice: description of the COOP Chart method and preliminary findings. *J Chronic Dis* 40 Suppl 1, 55S-69S.
- Nelson, E.C., Landgraf, J.M., Ron, D.H., Wasson, J.H., Kirk, J.W., 1990. The functional status of patients: How can it be measured in physicians' offices? *Med Care* 28, 1111-1126.
- O'Brien K., Nixon S., Tynan A.M., Glazier R.H., 2010. Aerobic exercise interventions for adults living with HIV/AIDS. *Cochrane Database Syst Rev* 4, 1-35.

- Palella, F.J., Deloria-Knoll, M., Chmiel, J.S., Moorman, A.C., Wood, K.C., Greenberg, A.E., Holmberg, S.D., the HIV Outpatient Study (HOPS) Investigators., 2003. Survival benefit of initiating antiretroviral therapy in HIV-infected persons in different CD4+ cell strata. *Ann Intern Med* 138, 620-626.
- Perna, F.M., LaPerriere, A., Klimas, N., Ironson, G., Perry, A., Pavone, J., Goldstein, A., Majors, P., Makemson, D., Talutto, C., Schneiderman, N., Fletcher, M.A., Meijer, O.G., Koppes, L., 1999. Cardiopulmonary and CD4 cell changes in response to exercise training in early symptomatic HIV infection. *Med Sci Sports Exerc* 31, 973-979.
- 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 Behav Immun* 19, 325-333.
- 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 Behav Immun* 20, 279-289.
- Phillips, A.C., 2011. Psychosocial influences on vaccine responses. *Soc Personal Psychol Compass* 5, 621-633.
- Pothoff, G., Wassermann, K., Ostmann, H., 1994. Impairment of exercise capacity in various groups of HIV-infected patients. *Respiration* 61, 80-85.
- Presanis, A.M., Gill, O.N., Chadborn, T.R., Hill, C., Hope, V., Logan, L., Rice, B.D., Delpech, V.C., Ades, A.E., De Angelis, D., 2010. Insights into the rise in HIV infections, 2001 to 2008: a Bayesian synthesis of prevalence evidence. *AIDS* 24, 2849-58.
- Ramírez-Marrero, F.A., Smith, B.A., Meléndez-Brau, N., Santana-Bagur, J.L., 2004. Physical and leisure activity, body composition, and life satisfaction in HIV-positive hispanics in Puerto Rico. *J Assoc Nurses AIDS Care* 15, 68-77.
- Ramírez-Marrero, F.A., Rivera-Brown, A.M., Nazario, C.M., Rodríguez-Orengo, J.F., Smit, E., Smith, B.A., 2008. Self-reported physical activity in hispanic adults living with HIV: Comparison with accelerometer and pedometer. *J Assoc Nurses AIDS Care* 19, 283-294.
- Remor, E., Penedo, F.J., Shen, B.J., Schneiderman, N., 2007. Perceived stress is associated with CD4+ cell decline in men and women living with HIV/AIDS in Spain. *AIDS Care* 19, 215-219.
- Schroeksadel, K., Sarcletti, M., Winkler, C., Mumelter, B., Weiss, G., Fuchs, D., Kemmler, G., Zangerle, R., 2008. Quality of life and immune activation in patients with HIV-infection. *Brain Behav Immun* 22, 881-889.

- Smit, E., Crespo, C.J., Semba, R.D., Jaworowicz, D., Vlahov, D., Ricketts, E.P., Ramírez-Marrero, F.A., Tang, A.M., 2006. Physical activity in a cohort of HIV-positive and HIV-negative injection drug users. *AIDS Care* 18, 1040-1045.
- Smith, B.A., Neidig, J.L., Nickel, J.T., Mitchell, G.L., Para, M.F., Fass, R.J., 2001. Aerobic exercise: effects on parameters related to fatigue, dyspnea, weight and body composition in HIV-infected adults. *AIDS* 15, 693-701.
- Smith, T.P., Kennedy, S.L., Fleshner, M., 2004. Influence of age and physical activity on the primary in vivo antibody and T cell-mediated responses in men. *J Appl Physiol* 97, 491-498.
- deSouza, P.M.L.d., Jacob-Filho, W., Santarqm, J.M., Silva, A.R.d., Li, H.Y., Burattini, M.N., 2008. Progressive resistance training in elderly hiv-positive patients: does it work? *Clinics* 63, 619-624.
- Stringer, W.W., Berezovskaya, M., O'Brien, W.A., Beck, C.K., Casaburi, R., 1998. The effect of exercise training on aerobic fitness, immune indices, and quality of life in HIV+ patients. *Med Sci Sports Exerc* 30, 11-16.
- Terry, L., Sprinz, E., Ribeiro, J.P., 1999. Moderate and high intensity exercise training in HIV-1 seropositive individuals: A randomized trial. *Int J Sports Med* 20, 142,146.
- Vaglio, J., Conard, M., Poston, W., O'Keefe, J., Haddock, C.K., House, J., Spertus, J., 2004. Testing the performance of the ENRICH social support instrument in cardiac patients. *Health Qual Life Outcomes* 2.
- Wallace, J.M., Rao, A.V., Glassroth, J., Hansen, N.I., Rosen, M.J., Arakaki, C., Kvale, P.A., Reichman, L.B., Hopewell, P.C., 1993. Respiratory illness in persons with human immunodeficiency virus infection. The pulmonary complications of HIV infection study group. *Am Rev Respir Dis* 148, 1523-1529.
- WHO., 2005. Recommendations for the production and control of pneumococcal conjugate vaccination.
- WHO., 2006. WHO Position Paper on Haemophilus influenzae type b conjugate vaccines.
- Woods, J.A., Keylock, K.T., Lowder, T.W., Vieira, V.J., Zelkovich, W., Dumich, S., Colantuano, K., Lyons, K., Leifheit, K.A., Cook, M., Chapman-Novakofski, K., McAuley, E., 2009. Cardiovascular exercise training extends influenza vaccine seroprotection in sedentary older adults: The immune function intervention trial. *Am J Chin Med* 57, 2183-2191.

## CHAPTER FIVE

### GENERAL DISCUSSION

#### **Summary of main findings**

The aim of this thesis was to investigate the effects of psychosocial factors and physical activity in healthy young, older, and HIV+ populations. Chapter Two found that an acute exercise intervention of 45 minutes of brisk walking did not enhance antibody response to pneumococcal or influenza vaccination in young (18-30 yrs) or older (50-64 yrs) adults. Although older participants had lower antibody responses to vaccination, compared to younger adults, they gained no additional benefits from the intervention. Chapter Three investigated whether a 16 week lifestyle physical activity intervention enhanced antibody response to pneumococcal vaccination in sedentary middle aged women. Although results showed that the intervention group significantly increased their walking behaviour and quality of life, compared to the control group, there were no differences between groups in any physiological measures or in pneumococcal antibody response at 4 weeks or 6 months post vaccination. However, when examining predictors of antibody response in all participants, higher levels of moderate physical activity at 4 week follow up and walking behaviour at 6 month follow up were associated with a greater antibody response at their respective time points. Finally, Chapter Four examined whether psychosocial factors and physical activity predicted antibody response to a glycoconjugate HIB and meningococcal C vaccine and pneumococcal vaccine in HIV+ patients. Results found that higher physical activity levels predicted a greater antibody response to pneumococcal strains 1, 6b and 18c at 4 weeks post vaccination. Furthermore, higher numbers of stressful life events and lower social support

predicted a lower antibody response to the pneumococcal 1 and 3 strains, respectively. However, the majority of analyses did not find a significant relationship, suggesting that psychosocial factors and physical activity status have limited associations with antibody response to vaccination in HIV+ patients.

### **Issue of intensity of moderate physical activity**

Chapters Two and Three employed moderate intensity interventions and found that walking behaviour did not augment antibody response to vaccination, whereas previous studies employing more intense interventions did improve antibody response (Edwards et al, 2006a; 2007; 2008; 2010; Kohut et al, 2004; Grant et al, 2008; Woods et al, 2009). Furthermore, vigorous and moderate intensity physical activity were significant predictors of some antibody strains in HIV+ patients, but no significant relationships were found when examining walking behaviour. It appears that, in all three studies, the intensity of physical activity may have important implications for its impact on immune function.

Progression of exercise intensity may also be important as to whether antibody response is improved. For example, one supervised physical activity intervention gradually increased the amount of physical activity participants carried out from 45-55%  $VO^2$  peak for 10-15 minutes to 60-70%  $VO^2$  peak for 45-60 minutes by the end of the intervention (Grant et al, 2008) and a previous acute exercise intervention used a progressive sub-maximal cycling protocol (Edwards et al, 2006a). It may be that if a moderate intensity intervention is utilised, a progressive component during the intervention is needed in order for an improvement in antibody response to be seen.

Moderate exercise may not be sufficient to induce the immune mechanisms which are required to enhance antibody response to vaccination. For example, a transient rise in IL-6 occurs during acute exercise (Edwards et al, 2006b); as higher levels of this cytokine have been associated with a higher antibody response following vaccination (Krakauer, 1995), this suggests that a brief increase in IL-6 may enhance subsequent vaccination response. In the first human behavioural adjuvant study, the IL6 response in the intervention groups significantly predicted antibody response to one of the influenza strains contained in the vaccine (Edwards et al, 2006a). In contrast, the acute study in this thesis employed a brief walking task; as walking is a habitual activity for most people (Bassett et al, 2008) and of relatively low intensity, it is unlikely to increase IL-6 levels (Markovitch et al, 2008). This may be one explanation for the lack of an adjuvant effect in this study.

Although a transient increase in IL6 may be beneficial, longer term elevations in proinflammatory cytokines may have a detrimental effect on antibody response. For example, those with higher visceral fat may have higher levels of proinflammatory adipokines, such as IL-6 and tumour necrosis factor (Gleeson et al, 2011), which could result in a less favourable immune environment for responding to pathogens (Cheung et al, 2011). The process of aging is also associated with an increased pro inflammatory state, and is characterised by poorer antibody responses to vaccination (Targonski et al, 2007). Physical activity can have an anti inflammatory effect upon this immune environment by reducing adiposity and the associated adipokines, such as IL-6 (Mujumdar et al, 2011; Ben Ounis et al, 2009). In our study, the lack of sufficient exercise intensity may explain why no changes in measures of adiposity, such as BMI and body fat percentage, were seen. Therefore, basal IL-6 levels were unlikely to have been reduced, which again may explain the lack of an effect on antibody response. This issue of intensity has particular relevance for the worldwide public health messages

which promote walking activity to maintain a healthy lifestyle (Haskell et al, 2007; Levine, 2007). These campaigns, which typically focus on walking behaviour (Lee and Buchner, 2008), should continue to promote the importance of increasing walking intensity in order to gain the clinical benefits, such as a decrease in adiposity.

### **Measurement of psychosocial and physical activity status**

Previous literature discussed in Chapter One demonstrated that both psychosocial factors and measures of physical activity are associated with antibody response to vaccination (Phillips, 2011; Senchina and Kohut, 2007). Examining both of these factors may particularly be pertinent as psychological stress and physical activity exert similar physiological responses, via the hypothalamic pituitary adrenal axis and sympathetic nervous system activation (Gleeson et al, 2011; Aguilera, 1998). Although some studies examining psychosocial factors have included basic measures of physical activity, and vice versa, few have looked at their interactions. However, one study examined this in more detail and found that psychological factors partly mediate the relationship between increasing physical activity levels and antibody response (Kohut et al, 2005). In addition, research from this thesis highlights the importance of considering the impact of both psychosocial factors and physical activity when examining antibody response to vaccination. For example, in Chapter Three, the intervention group experienced both an increase in physical activity levels and an improvement in quality of life, which suggests that changing exercise behaviour may also improve psychological status. It is possible that exercise improves psychosocial factors, and this reduction in stress levels makes it easier to adhere to the exercise regime. Therefore, both factors should be examined in detail when carrying out studies looking at antibody response to vaccination.



## **Type of vaccination**

Thymus dependent and independent vaccinations stimulate different immune pathways which can give an indication of the types of immune cells that are affected by psychosocial factors or physical activity. For example, Chapter Three suggests that the T independent pathway is not affected by increasing physical activity, but it is not clear whether this would be the case for a T dependent vaccination, such as influenza. As previous studies have found higher antibody responses with T dependent antigens (Kohut et al, 2004; Grant et al 2008; Woods et al, 2009), it is possible that physical activity is more likely to affect T dependent antigens in healthy adults, although this has not yet been directly compared. Conversely, in Chapter Four, some physical activity and psychosocial variables were associated with T independent, but not T dependent antibody response. Therefore, it is likely that psychosocial factors and physical activity levels are not limited to one particular type of vaccine. However, further studies should still consider using a variety of vaccine types to further elucidate which immune pathways are affected by these psychosocial and physical activity variables.

## **Timing of the intervention**

The timing of the physical activity intervention in Chapters Two and Three in relation to the vaccination may also affect antibody response. During moderate intensity exercise, some of the acute physiological changes are relatively small and short-lived; for example, there is likely to be only an acute increase in lymphatic drainage during exercise, which could speed up clearance of the antigen to the lymph nodes, thus improving immune response (Havas et al, 1997). Furthermore, the recruitment of leukocytes into the blood stream seen during exercise (Shek et al, 1995) may also improve antibody response due to the higher numbers of immune

cells readily available to respond to antigen; these cells rapidly return to marginal pools following exercise cessation (Anane et al, 2009; Campbell et al, 2009). Overall, this means that it is likely to be important to carefully time the intervention in relation to the vaccination. As such, it would be useful to examine whether completing the exercise protocol prior to the vaccination would be more effective at improving antibody response.

In the chronic training study (Chapter Three), the vaccination was administered three months into the intervention, whereas previous physical activity interventions were carried out for between 8 and 10 months prior to vaccination (Kohut et al, 2004; Grant et al, 2008; Woods et al, 2009). Furthermore, one review concluded that significant weight loss was achieved in interventions of at least 6 months duration, but not those of a shorter duration (Catenacci and Wyatt, 2007). It is possible that a longer intervention is needed in order for physiological and immunological changes to take effect. Therefore, these are plausible suggestions as to why the timing of the vaccination in relation to the physical activity intervention did not affect antibody response in Chapters Two or Three.

Previous literature examining physical activity and HIV progression suggests that there may also be a difference between studies conducted before and after the advent of antiretroviral therapy (ART), which maintains CD4+ counts and dramatically prolongs a healthy life. For example, in the pre ART era, higher physical activity levels tended to be associated with a slower HIV progression (Pothoff et al, 1994; LaPerriere et al, 1991; Perna et al, 1999), whereas in the post ART era, fewer studies have shown a positive relationship between physical activity and HIV status (Bopp et al, 2004, deSouza et al, 2008). This suggests that those who are on ART and have relatively high and stable CD4+ counts are not affected by behavioural factors, such as physical activity. The majority of HIV+ patients in our participant cohort were on ART medication, but we only found small effects in a minority of

strains. It may be that psychosocial factors and physical activity behaviour have stronger and more associations with antibody response when CD4+ count is lower, perhaps prior to taking ART, or at an advanced stage of illness.

### **Future studies**

As a key theme in the discussion is the issue of intensity, it would be interesting to test whether a higher intensity walk, involving primarily uphill walking, prior to vaccination would have an effect upon antibody response to vaccination. As IL-6 response tends to only occur during more intense physical activity (Markovitch et al, 2008), uphill walking may be sufficient to elicit this IL-6 response, and therefore antibody response to vaccination may be augmented. Although a higher intensity uphill walk may be less applicable to a clinical setting, it may help elucidate whether exercise intensity is a significant factor in behavioural adjuvant research.

In regard to chronic physical activity interventions, as we found that physical activity levels predicted antibody response at 4 weeks and 6 months in all participants, it is possible that antibody response is affected by another factor related to physical activity, such as reductions in visceral fat. Supplementary analyses in Chapter Three showed that a higher BMI and body fat percentage were associated with lower walking behaviour. Therefore, it is possible that measures of adiposity, via a reduction in adipokines (Gleeson et al, 2011), may mediate the relationship between physical activity and augmentation of antibody response. Furthermore, body fat percentage was measured using bioelectrical impedance, which is a valid and reliable measure of body fat percentage, but can be confounded by hydration status, skin temperature and the menstrual cycle (Brodie et al, 1998). This suggests that further studies examining

physical activity interventions and immune function warrant a more accurate measurement of body adiposity, such as Dual-emission X-ray absorptiometry.

Although only a small number of significant findings were found in Chapter Four, physical activity was a significant predictor for three of the pneumococcal strains. Furthermore, supplementary analyses found that higher vigorous physical activity levels predicts higher social support and quality of life, which indicates that physical activity may be beneficial for psychological health. A lifestyle physical activity intervention study may be appropriate to examine whether improving physical activity levels in HIV+ patients would also improve antibody response, especially as this would deal with unreliable estimates of exercise; HIV+ patients tend to over-report the amount of physical activity which they carry out (Fillipas et al, 2010) and a physical activity intervention, which is compared to a control group, would help remove the implications of this recall bias. Furthermore, a previous lifestyle physical activity intervention found that participants had stronger associations between their objective and subjective measures of physical activity at follow up, compared to at baseline (Long et al, in preparation). This implies that those partaking in a physical activity intervention may become more aware of how much physical activity they carry out, and therefore a more accurate measure of physical activity is attained. Previous physical activity interventions in HIV+ patients have suffered from high attrition rates, low power and tend to attract a homogenous population sample of 18-58 year old males (O'Brien et al, 2010). A unsupervised lifestyle physical activity intervention may be an appropriate method to increase physical activity within the HIV+ population, particularly as walking behaviour is an accessible and preferred mode of exercise for women (Salmon et al, 2003). However, this intervention would need to address the limitations mentioned in Chapter Three, such as the intensity of the exercise, duration of the intervention and type of vaccinations used. For example, a second

consultation emphasising increasing exercise intensity could be given to the participant and a longer duration of the intervention period may allow more time for physiological and immunological changes to occur. Participants could also be given an accelerometer, which measures exercise intensity and energy expenditure (Troiano, 2006), which would help them monitor and increase their physical activity intensity throughout the intervention. Overall, an exercise trial of this nature would elucidate whether a practical lifestyle intervention could be successfully completed by HIV+ patients, and if it has any physiological, psychological and immunological benefits.

As HIV+ patients achieved relatively poor antibody responses to the vaccinations in Chapter Four, a study investigating whether a behavioural adjuvant improves vaccination responses could also be carried out. Although Chapter Two found that a brisk walk did not improve antibody response to vaccination, previous research using healthy participants has found that moderate cycling and resistance exercise can enhance antibody response, and this is particularly effective when the control response is poor (Edwards et al, 2006a; 2007; 2008; 2010). As HIV+ patients are immunocompromised and have poor antibody responses to vaccination, future research could use an eccentric exercise intervention to investigate whether antibody response can be improved in this immune compromised population.

## **CONCLUSION**

This thesis examined whether psychosocial factors and physical activity affected antibody response to vaccination in young, older and HIV+ populations. Neither an acute or chronic moderate intensity physical activity intervention affected antibody response to vaccination (Chapters Two and Three). However, there was some limited evidence that psychosocial status and physical activity status did predict antibody response to the pneumococcal vaccination in both sedentary healthy and HIV+ participants. As previous research has demonstrated that higher intensity aerobic exercise, in both acute and chronic doses, can be an effective vaccine adjuvant (Edwards et al, 2006a, Kohut et al, 2004; Grant et al, 2008; Woods et al, 2009), these findings suggest that the intensity of the physical activity intervention may be an important determinant of the effect of exercise on antibody response to vaccination.

## REFERENCES

- Aguilera, G., 1998. Corticotropin releasing hormone, receptor regulation and the stress response. *Trends Endocrinol Metab* 9, 329-36.
- Anane, L.H., Edwards, K.M., Burns, V.E., Drayson, M.T., Riddell, N.E., van Zanten, J.J.C.S., Wallace, G.R., Mills, P.J., Bosch, J.A., 2009. Mobilization of CD8+ T lymphocytes in response to psychological stress, exercise, and beta agonist infusion. *Brain, Behavior, and Immunity* 23, 823-829.
- Bassett, D.R., Pucher, J., Buehler, R., Thompson, D.L., Crouter, S.E., 2008. Walking, cycling, and obesity rates in Europe, North America, and Australia. *J Phys Act Health* 5, 795-814.
- Ben Ounis, O., Elloumi, M., Lac, G., Makni, E., Van Praagh, E., Zouhal, H., Tabka, Z., Amri, M., 2009. Two-month effects of individualized exercise training with or without caloric restriction on plasma adipocytokine levels in obese female adolescents. *Ann Endocrinol (Paris)* 70, 235-41.
- Bopp, C.M., Phillips, K.D., Fulk, L.J., Dudgeon, W.D., Sowell, R., Hand, G.A., 2004. Physical activity and immunity in HIV-infected individuals. *AIDS Care* 16, 387-393.
- Brodie, D., Moscrip, V., Hutcheon, R., 1998. Body composition measurement: A review of hydrodensitometry, anthropometry, and impedance methods. *Nutrition* 14, 296-310.
- Campbell, J.P., Riddell, N.E., Burns, V.E., Turner, M., van Zanten, J.J.C.S., Drayson, M.T., Bosch, J.A., 2009. Acute exercise mobilises CD8+ T lymphocytes exhibiting an effector-memory phenotype. *Brain, Behavior, and Immunity* 23, 767-775.
- Catenacci, V.A., Wyatt, H.R., 2007. The role of physical activity in producing and maintaining weight loss. *Nat Clin Pract Endocrinol Metab* 3, 518-529.
- Cheung, K.P., Taylor, K.R. Jameson, J.M., 2011. Immunomodulation at epithelial sites by obesity and metabolic disease. *Immunol Res* [Epub ahead of print].
- Souza, P.M., Jacob-Filho, W., Santarém, J.M., Silva, A.R., Li, H.Y., Burattini, M.N., 2008. Progressive resistance training in elderly hiv-positive patients: does it work? *Clinics* 63, 619-624.
- Edwards, K.M., Burns, V.E., Reynolds, T., Carroll, D., Drayson, M., Ring, C., 2006a. Acute stress exposure prior to influenza vaccination enhances antibody response in women. *Brain Behav Immun* 20, 159-168.
- Edwards, K.M., Burns, V.E., Ring, C., Carroll, D., 2006b. Individual differences in the interleukin-6 response to maximal and submaximal exercise tasks. *J Sports Sci* 24, 855-862.

- Edwards, K.M., Burns, V.E., Allen, L.M., McPhee, J.S., Bosch, J.A., Carroll, D., Drayson, M., Ring, C., 2007. Eccentric exercise as an adjuvant to influenza vaccination in humans. *Brain Behav Immun* 21, 209-217.
- Edwards, K.M., Burns, V.E., Adkins, A.E., Carroll, D., Drayson, M., Ring, C., 2008. Meningococcal A vaccination response is enhanced by acute stress in men. *Psychosom Med* 70, 147-151.
- Edwards, K.M., Campbell, J.P., Ring, C., Drayson, M.T., Bosch, J.A., Downes, C., Long, J.E., Lumb, J.A., Merry, A., Paine, N.J., Burns, V.E., 2010. Exercise intensity does not influence the efficacy of eccentric exercise as a behavioural adjuvant to vaccination. *Brain Behav Immun* 24, 623-630.
- Fillipas, S., Bowtell-Harris, C.A., Oldmeadow, L.B., Cicuttini, F., Holland, A.E., Cherry, C.L., 2008. Physical activity uptake in patients with HIV: who does how much? *Int J STD AIDS* 19, 514-518.
- Gleeson, M., Bishop, N.C., Stensel, D.J., Lindley, M.R., Mastana, S.S., Nimmo, M.A., 2011. The anti-inflammatory effects of exercise: mechanisms and implications for the prevention and treatment of disease. *Nat Rev Immunol* 11, 607-615.
- 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 Behav Immun* 22, 923-932.
- Haskell, W.L., Lee, I.M., Pate, R.R., Powell, K.E., Blair, S.N., Franklin, B.A., Macera, C.A., Heath, G.W., Thompson, P.D., Bauman, A., 2007. Physical activity and public health: Updated recommendation for adults from the American College of Sports Medicine and the American Heart Association. *Med Sci Sports Exerc* 39, 1423-34.
- Havas, E., Parviainen, T., Vuorela, J., Toivanen, J., Nikula, T., Vihko, V., 1997. Lymph flow dynamics in exercising human skeletal muscle as detected by scintigraphy. *J Physiol* 504, 233-239.
- Kohut, M.L., Lee, W., Martin, A., Arnston, B., Russell, D.W., Ekkekakis, P., Yoon, K.J., Bishop, A., Cunnick, J.E., 2005. The exercise-induced enhancement of influenza immunity is mediated in part by improvements in psychosocial factors in older adults. *Brain Behav Immun* 19, 357-366.
- Kohut, M.L., Arntson, B.A., Lee, W., Rozeboom, K., Yoon, K.J., Cunnick, J.E., McElhaney, J., 2004. Moderate exercise improves antibody response to influenza immunization in older adults. *Vaccine* 22, 2298-2306.
- Krakauer, T., 1995. Levels of interleukin 6 and tumor necrosis factor in serum from humans vaccinated with live, attenuated *Francisella tularensis*. *Clin. Diagn. Lab. Immunol.* 2, 487-488.



- LaPerriere, A., Fletcher, M.A., Antoni, M.H., Klimas, A., Ironson, G., Schneiderman, N., 1991. Aerobic exercise training in an AIDS risk group. *Int J Sports Med* 12, S53-S57.
- Lee, I., Buchner, D.M., 2008. The importance of walking to public health. *Med Sci Sports Exerc* 40, S512-S518.
- Levine, J.A., 2007. Exercise: A Walk in the Park? *Mayo Clin Proc* 82, 797-798.
- Long, J.E., Ring, C., Bosch, J.A., Eves, F., Drayson, M., Calver, R., Say, V., Allen, D., Burns, V.E., A lifestyle physical activity intervention and the antibody response to pneumococcal vaccination in women. In preparation.
- Markovitch, D., Tyrrell, R.M., Thompson, D., 2008. Acute moderate-intensity exercise in middle-aged men has neither an anti- nor proinflammatory effect. *J Appl Physiol* 105, 260-265.
- Mujumdar, P.P., Duerksen-Hughes, P.J., Firek, A.F., Hessinger, D.A., 2011. Long-term, progressive, aerobic training increases adiponectin in middle-aged, overweight, untrained males and females. *Scand J Clin Lab Invest* 71, 101-107.
- O'Brien K., Nixon S., Tynan A.M., Glazier R.H., 2010. Aerobic exercise interventions for adults living with HIV/AIDS. *Cochrane Database Syst Rev* 4, 1-35.
- Perna, F.M., LaPerriere, A., Klimas, N., Ironson, G., Perry, A., Pavone, J., Goldstein, A., Majors, P., Makemson, D., Talutto, C., Schneiderman, N., Fletcher, M.A., Meijer, O.G., Koppes, L., 1999. Cardiopulmonary and CD4 cell changes in response to exercise training in early symptomatic HIV infection. *Med Sci Sports Exerc* 31, 973-979.
- Phillips, A.C., 2011. Psychosocial influences on vaccine responses. *Soc Personal Psychol Compass* 5, 621-633.
- Pothoff, G., Wassermann, K., Ostmann, H., 1994. Impairment of exercise capacity in various groups of HIV-infected patients. *Respiration* 61, 80-85.
- Salmon J, Owen N, Crawford D, Bauman A, Sallis JF. 2003 Physical activity and sedentary behavior: a population-based study of barriers, enjoyment, and preference. *Health Psychol* 22, 178-88.
- Senchina, D.S., Kohut, M.L., 2007. Immunological outcomes of exercise in older adults. *Clin Interv Aging* 2, 3-16.
- Shek, P.N., Sabiston, B.H., Buguet, A., Radomski, M.W., 1995. Strenuous exercise and immunological changes. *Int J Sports Med* 16, 466,474.
- Targonski, P.V., Jacobson, R.M., Poland, G.A., 2007. Immunosenescence: role and measurement in influenza vaccine response among the elderly. *Vaccine* 25, 3066-3069.

- Troiano, R.P., 2006. Translating accelerometer counts into energy expenditure: advancing the quest. *J Appl Physiol* 100, 1107-1108.
- Woods, J.A., Keylock, K.T., Lowder, T.W., Vieira, V.J., Zelkovich, W., Dumich, S., Colantuano, K., Lyons, K., Leifheit, K.A., Cook, M., Chapman-Novakofski, K., McAuley, E., 2009. Cardiovascular exercise training extends influenza vaccine seroprotection in sedentary older adults: The immune function intervention trial. *Am J Chin Med* 57, 2183-2191.