SUBJECTIVE WELL-BEING AND BIOMARKERS OF HEALTH

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ABSTRACT

An association between inflammation and mood deterioration has been proposed as a potential explanatory mechanism underlying many pathologies. Previous research attributes this consistently reoccurring connection between inflammation and psychopathology that is often reported within the literature, to a relationship between the HPA axis, the body’s stress response system and the immune system. There is evidence of a bidirectional feedback loop between end-products of the immune system and the HPA-axis such as cytokines and cortisol. This is supported by research reporting that components of subjective well-being such as positive affect, optimism and life satisfaction can produce beneficial health outcomes by potentially targeting this feedback loop. The present longitudinal study tested if higher positive affect independently corresponds to lower levels of inflammatory markers Interleukin-6 (IL-6) and C-reactive protein (CRP) and HPA axis marker cortisol. The study further tested if higher subjective well-being decreases levels of IL-6 and CRP as well as cortisol. The study employed a subsample of participants from the Midlife in Japan (MIDJA) Biomarker project (n=174) that underwent testing at two separate time points across a period of 4 years. The data included subjective well-being, positive affect, IL-6, CRP, cortisol, perceived stress, neuroticism and demographic variables. Positive affect was not associated with any inflammatory marker or cortisol. Subjective well-being had no effect on CRP but reduced IL-6 and cortisol even when controlling for all control and demographic variables. It is concluded that subjective well-being may be linked to lower inflammation and HPA axis activity.

Keywords: Subjective well-being, Positive affect, biomarkers, inflammatory cytokines, HPA axis, Interleukin 6, C-reactive protein, Cortisol

ABSTRAKT

Ett samband mellan inflammation och sjukdomsbeteende har föreslagits som en förklaringsmekanism bakom förekomsten av många patologier. Den konsekventa anknytningen mellan inflammation och psykopatologi som många tidigare studier demonstrerat innebär ett samband mellan immunystemet och HPA-axeln som är den struktur som utgör kroppens svar på stressorer. Det finns tecken på att en återkopplingslänka mellan slutprodukter av det immunologiska systemet och HPA-axeln såsom cytokiner och kortisol. Detta har stöd i tidigare forskning som rapporterat att komponenter av subjektivt välbefinnande såsom positiv affekt, optimism och livstillfredsställelse kan medföra positiva hälsoutfall genom att potentiellt infliura denna återkopplingslänka. Förevarande longitudinala studie testar om högre positiv affekt leder till lägre nivåer av de inflammatoriska markörerna interleukin-6 (IL-6) och C-reaktivt protein (CRP) samt HPA-axel markören kortisol. Studien testar vidare även om högre subjektivt välbefinnande leder till lägre nivåer av IL-6, CRP och kortisol. Deltagarna är ett subsampel från Biomarkerprojektet (n = 174) inom Midlife in Japan (MIDJA) som genomgick testning vid två separata tidpunkter över en period av 4 år. Data består av subjektivt välbefinnande, positiv affekt, IL-6, CRP, kortisol, upplevd stress, neuroticism samt demografiska variabler. Positiv affekt hade ingen signifikant effekt på någon av de inflammatoriska markörerna eller kortisol. Subjektivt välbefinnande hade inte någon signifikant effekt på CRP men reducerade signifikant IL-6 och kortisol och dessa effekter förblev signifikanta efter kontroll för samtliga
kontroll och demografiska variabler. Följaktligen dras slutsatsen att subjektivt välbefinnande kan leda till lägre inflammation och HPA-axel aktivitet.

Nyckelord: Subjektivt välbefinnande, Positiv affekt, biomarkörer, inflammatoriska cytokiner, HPA-axeln, Interleukin-6, C-reaktivt protein, Kortisol
The past two decades has seen an increase in studies from diverse areas of neuroscience, immunology and epidemiology reporting that chronic stress is a reliable predictor of systemic inflammation in the absence of pathogens. Conventional beliefs regarding inflammation are strictly related to pathogenic stimuli as an activator for inflammatory processes. These assumptions are now being questioned by a growing body of evidence that suggests that non-immunological stimuli may hold equal potential to engender systemic inflammation (Yang et al., 2014). In addition, inflammation is being heralded as a potential pathway underlying the relationship between stress experience and health (Strahler, Rohleder & Wolf, 2014). The association between psychological, behavioral and environmental factors and immunity appears to rely heavily upon two separate yet co-operating structures in the form of the Hypothalamic-Pituitary Adrenal (HPA) axis, the body’s neuroendocrine system that regulates and controls responses to stressors and the immune system that defends the body from pathogens. Contemporary research proposes that whenever a dysregulation occurs within either one of these structures physical or psychological pathology can arise as a consequence. There is evidence of a bidirectional feedback loop between end-products of the immune system and the HPA axis, such as cortisol and cytokines. This feedback loop is central for the functioning of the HPA axis while also maintaining homeostasis of the immune system (Wolkow, Aisbett, Reynolds, Fergusson & Main, 2015). The relationship between psychological factors and inflammation may however also rely upon neurotransmitter dopamine. Indeed, the effect that inflammatory markers induce upon dopamine and the basal ganglia are considered relevant for elevating depressive symptoms, indicating that dopamine is affected when inflammatory cytokines increase within the body (Felger, 2017). In addition to this, dopamine is also reported to possess an anti-inflammatory effect rendering the relationship bidirectional in nature (Yan et al., 2015).

A great deal of evidence in favor of the bidirectional relationship between the HPA axis and the immune system stems from studies with clinical populations. For instance it has been reported that suicide attempters oftentimes suffer from blunted HPA axis activity as well as heightened levels of inflammatory markers (Melhem et al., 2017). Elevated levels of inflammatory cytokines are reported in major depressive disorder (MDD) (Karlovic, Serretti, Vrkić, Martinac & Marčinko, 2012), anxiety (O’Donovan et al., 2010), schizophrenia and bipolar disorder (Shi et al., 2009) and in adults with a history of childhood adversity such as neglect or abuse (Measelle, David & Ablow, 2016). In addition chronic stress has been reported as a cause of neuroinflammation (Cheng et al., 2016). Functional brain studies report that systemic inflammation brings about mood-dependent changes in the subgenual anterior cingulate cortex (sACC) thereby rendering inflammation associated changes within total mood reliant upon neural circuitry similar to that of true depression (Harrison et al., 2009).

As the relationship between the immune system and the HPA axis is implemented in much medical and psychological pathology there is an increasing need for factors that can target this bidirectional loop and aid in better health outcomes. Studies have reported that positive affect, life satisfaction, and optimism act as buffer agents upon inflammation and stress (Stellar et al., 2015; Segerström & Sephton, 2010). Anti-inflammatory attributes of positive affect have been reported in chronic heart failure patients (Brouwers et al., 2012), in individuals with high positive emotional style (Dockray & Steptoe, 2010), in high stress
individuals (Okely et al., 2016) and arthritis patients (David et al., 2008). Trait positive affect is further associated with lower production of inflammatory cytokines (Prather et al., 2007). Indeed a lack of positive emotions appears highly relevant for systemic inflammation (Deverts et al., 2010). Even minor daily experiences can buffer against stress as daily positive events were found to act as a protective agent against systemic inflammation (Sin, Graham-Engeland & Almeida, 2015). Positive affect, optimism and life satisfaction are components of the construct of subjective well-being (Gale, Booth, Mottus, Kuh & Deary, 2013). As these factors possess an anti-inflammatory effect independently the question arises as to whether a collective effect through subjective well-being could target the bidirectional feedback loop between the immune system and the HPA axis to a greater extent. This could potentially restore homeostasis, thus reversing the effects and relieving the symptomology related to a dysregulated HPA axis and immune response by decreasing levels of inflammatory and HPA markers within the systems. Given the lack of knowledge regarding the potential benefactor effect of subjective well-being the current study aims to investigate whether it can impact biomarkers of immune function and HPA axis activation. Subjective well-being is not fixed and can be altered through psychotherapy and cognitive restructuring (Ironson et al., 2016), and could thereby potentially aid clinicians and patients in procuring better psychological and medical health outcomes.

In general the literature in this area has focused primarily on the impact of negative affect and psychopathology upon the immune system, notwithstanding that this association may be influenced by cultural factors. This is relevant to draw attention to as a recent study failed to confirm the link between negative affect and inflammatory biomarkers in Japanese individuals but was successful in establishing this relationship in an American sample (Miyamoto et al., 2013). For this reasons the present study also seeks to understand whether the association between positive psychological factors and health reported within American samples could be replicated in Japan.

**The Hypothalamic-pituitary adrenal axis and cortisol**

The Hypothalamic-pituitary adrenal axis (HPA axis) is one of two identified stress axes and alterations within this axis are regularly linked to the development of mood related disorders. The axis is composed of three components; the hypothalamus, the pituitary gland and the adrenal gland and is responsible for mounting a response to stress. Sensitization of the HPA axis, following chronic stress or stress due to early life adversities is associated with a heightened risk of developing depression. Moreover research linking chronic psychological stressor to psychiatric and cardiometabolic disorder is accumulating, implementing HPA axis dysregulation as the underlying mechanism (Spanakis, Wand, Ji & Golden, 2015). The HPA axis is stimulated by all forms of stress and will upon activation initiates secretion of corticotrophin releasing hormone (CRH), and arginine vasopressin (AVP) which together bring about the release of adrenocorticotropic hormone (ACTH) from the anterior pituitary (Spanakis, Wand, Ji & Golden, 2015). ACTH circulates through the blood and prompts the adrenal medulla to create cortisol. In natural conditions cortisol helps the body handle stress, however if we fail to cope alternatively if the stress is too extensive, excessive amounts of cortisol within the system can have a negative impact upon the brain thereby damaging feedback loops that the brain utilizes in cessation of the stress response (Kolb & Whishaw, 2015).

Cortisol is considered to be the final product of the HPA axis and is commonly accepted as a main predictor of an individual’s level of cumulative physiological risk related to chronic stress. The secretion of cortisol from the HPA axis follows a diurnal rhythm with
higher levels at awakening that peak in the following thirty minutes only to decline progressively throughout the day, this process is identified as the cortisol slope. A cortisol circadian profile that is flattened has been identified as having negative impact upon physical health (Kumari et al., 2011; Kumari, et al., 2010). Excessive stress due to early life adversities has moreover been found to be exceptionally detrimental for future life outcomes. If abuse, neglect or other severe environmental stress occurs during the critical periods in early childhood it can permanently disrupt the reactivity of the HPA axis resulting in a constantly active stress axis. This dysregulation within the HPA axis leads to the over-secretion of cortisol, a condition closely related to depression in adulthood (McGowan et al., 2010). The HPA axis is thus responsible for the development of mood related disorders and cortisol is connected to negative health outcomes. Inflammation is being heralded as a potential pathway underlying the relationship between stress experience and health (Strahler, Rohleder & Wolf, 2014), and from this the question arises whether the immune system and the HPA axis may be connected.

The immune system, inflammatory cytokines and C-reactive protein

Identified as a primary pathophysiological mechanism driving life-threatening conditions, systemic inflammation represents a major factor in the development and progression of human disease. Inflammatory processes have previously been considered as exclusively triggered by infectious stimuli but new evidence indicates that non-immunological stimuli may hold equal potential to bring about systematic inflammation. Chronic stress is being reported as a significant predictor of systemic inflammation in the absence of pathogenic invasion or tissue damage.

An inflammatory response is a natural biological response to a pathogen or physical damage and a vital function of the immune system (Measelle et al., 2016). The process is organized through different forms of inflammatory mediators with the aim of restoring tissue damage or resolving infection without overshooting in inflammation. Should dysfunction arise within this system or if the individual is exposed to repeated presence of stimulatory agents or chronic stress there is a risk of chronic inflammation which damages local tissue and ultimately results in pathologies increasing the risk for chronic illness (Yang et al., 2014; Hänsel et al., 2010). The inflammatory response is organized by multi-functional pro-inflammatory cytokines which initiate synthesis of acute phase reactant C-reactive protein (CRP) that aids in the removal and restoration of damaged cells. CRP has a short lifespan rendering circulating levels of CRP within the blood reliable biomarkers of chronic inflammation. Research indicates that chronically elevated levels of CRP are prognostic of medical as well as psychiatric disease (Measelle et al., 2016). CRP is identified as a protein synthesized in the liver as a response to components released by macrophages and fat cells like adipocytes. It is defined as an acute phase protein and is regulated by pro-inflammatory cytokines such as IL-6 that are activated early on in the inflammatory process (Halaris, 2016).

A crucial aspect of the immune system is thus represented by inflammatory cytokines. These components of the immune system are cell signaling proteins that mediate and regulate the immune response. These cytokines have been implied as important key parameters in many etiopathological mechanisms as well as impaired neuroplasticity (Liu, Adibfar, Herrmann, Gallagher & Lanctot, 2016). Defined as regulatory glycoproteins, cytokines are produced by many different cell types of which leukocytes are a major source. Levels of circulating cytokines in the blood are suitable markers of inflammatory conditions as they are produced by activated immune cells and aid in activating other cells which consequently bring about further synthesis and actions of even further cytokines during inflammation.
Certain cytokines, such as IL-6, appear responsive to psychosocial stimuli and further influence psychological states and behaviors (Hänsel et al., 2010). Pro-inflammatory peripheral blood cytokine IL-6 brings about depressive symptoms through a multitude of processes related to affect. Increase in inflammatory signaling further impairs neuronal health, dysregulates neurotransmitter metabolism and alters neural activity within affectivity related brain regions (Miller et al., 2013; Slavich & Irwin, 2014). Inflammatory cytokines have moreover been reported as contributors to oxidative stress, thereby damaging glial cells in brain regions relevant for affect such as the amygdala and prefrontal cortex (Leonard & Maes, 2012) and as promoting dysregulation within the HPA axis (Stetler & Miller, 2011).

Elevated levels of IL-6 and CRP are oftentimes ascribed as systemic inflammation as they lend insight into a non-specific ongoing inflammatory process within the body (Yang et al., 2014). Systemic inflammation is however not restricted to pathogens and tissue damage. A growing body of research identifies psychological stress, acute as well as chronic, as associated with systemic inflammation (Rohleder, 2014; Goldstein, 2015).

Biological theory has suggested that the association between systemic inflammation and psychological distress is bidirectional in nature and has further proposed distress as a mediator rather than a precursor of the link between inflammation and morbidity. According to this perspective there exists a direct psychobiological pathway from distress to inflammation. This response overrides downregulation of anti-inflammatory processes and induces inflammatory reactions (Das, 2016). Animal and human studies report that depression induces inflammatory reaction within the system through increase in inflammatory cytokines in regards to reactivity to stressors (Carpenter et al., 2010). Inversely elevated levels of inflammatory markers have been found to induce depression (Valkanova et al., 2013). By this nature it then follows that the immune system responds to stress with heightened inflammation and contrariwise brings about mood deterioration when inflammation is present within the body. These effects appear to rely heavily upon pro-inflammatory cytokine IL-6 that emerges as a crucial component in elevating mood symptoms. As the HPA axis is responsible for mood symptoms and stress regulation it appears that these two systems may be connected to one another.

The relationship between the immune system and the HPA axis

The relationship between the HPA axis and the immune system appears convincing but was previously considered weak as glucocorticoids like cortisol are known to have an anti-inflammatory effect. Recent findings have however reported that although serum cortisol is elevated in response to cytokines the anti-inflammatory effect that it produces is low as a result of the cytokine induced decrease in glucocorticoid receptor synthesis, translocation and binding. As the HPA axis becomes dysregulated and inflammatory levels increase within the system the systemic inflammation has been found to impair neuroplasticity and thereby lead to structural and functional brain changes (Rosenblat et al., 2014). There is evidence of a bidirectional feedback loop between end-products of the immune and endocrine systems such as cortisol and cytokines. This feedback loop is central for the functioning of the HPA axis while also maintaining homeostasis of the immune system. Chronic stressors causing a prolonged activation of the HPA axis results in excessive cortisol release contributing to inflammation as excessive cortisol impairs functioning of glucocorticoid receptors. These abnormalities subsequently decreases the immune system’s ability to respond to cortisol and lower the inflammation thereby leaving cortisol and cytokines at sustained release levels (Wolkow, Aisbett, Reynolds, Fergusson & Main, 2015). Cytokines can thus stimulate
activation of the HPA axis and contrariwise cortisol can consequently bring about an inflammatory response.

Indeed, inflammatory cytokines are known to successfully activate the HPA axis bringing about a significant inflation in the production of glucocorticoids with high anti-inflammatory potency. Older studies have reported that IL-6 successfully induces an increase in plasma cortisol (Steensberg, Fischer, Keller, Moller & Klarlund Pedersen, 2003; Weber, Auernhammer & Engelhardt, 1997). This evidence is often taken as indicative of that cytokine-induced HPA axis hyperactivity is a key factor in the development of symptom dimension of mood and cognition associated with depression. It is believed that the impact of pro-inflammatory cytokines upon the HPA axis may induce gene expression and synthesis of corticotrophin release factor (CRF) which stimulates ACTH release and fundamentally causes glucocorticoid secretion (Baune, 2016), this effect is considered a well-established component of a more general stress response resulting from inflammation (Rosenblat et al., 2014). HPA axis activation can lead to a further increase in pro-inflammatory cytokines through a positive feedback loop in which stress can bring about cytokine level increase (Baune, 2016). Pro-inflammatory cytokines are further linked to decrease in expression, translocation and downstream effects of glucocorticoid receptors consequently blunting the negative feedback loop of the HPA axis and ultimately allowing for further increase in cortisol level (Pace & Miller, 2009). These cytokines bring about a disproportionate increase in HPA axis activity in comparison to the normal response resulting in inflated cortisol levels through the cytokine stimulation of the HPA axis and by the impaired HPA negative feedback self-regulation. These elevated glucocorticoid levels are frequently reported as resulting in mood symptoms and inducing a pro-inflammatory state in medically ill or healthy individuals will reliably increase serum levels of pro-inflammatory cytokines and subsequently increase prevalence of mood symptoms (Rosenblat et al., 2014). Evidence imply that there is an increased risk for suicidal behavior following elevated inflammation for individuals with hepatitis C, cancer and multiple sclerosis (Frasogo et al., 2010), autoimmune disorders (Fuller-Thomson et al., 2016; Ludvigsson et al., 2011), infections (Brundin and Grit, 2016; Lund-Sorensen et al., 2016) and allergies and asthma (Goodwin, 2012). HPA axis hypo-activity is found within many allergic disorders (Trueba et al., 2016) and the connection between depression and cardio-metabolic outcomes could be mediated through inflammation (Chirinos, Murdock, LeRoy & Fagundes, 2017; Vogelzangs et al., 2011). Inflammation has also been linked to anxiety (O’Donovan et al., 2010), impulsivity and aggression (Coccaro et al., 2014; Isung et al., 2014), sleep problems (Prather et al., 2009) and a 4-fold increase in suicidal risk (Batty et al., 2016). Systemic inflammation triggers behavioral changes in the form of “sickness behavior” characterized by mood symptoms, changes in motivation and sleep disturbances among others that show a great resemblance to those of major depressive disorder and could therefore potentially explain the high rates of depression within medically ill patients (Harrison et al., 2009).

Contrariwise the HPA axis stimulation of the immune systems begins when the individual faces a psychological stressor and the hypothalamic paraventricular nucleus integrates and secretes CRH into the pituitary portal vein system. CRH then stimulates the pituitary to produce ACTH into the systemic circulation which ultimately brings about the production of glucocorticoids, primarily cortisol from the adrenal cortex. Physiological doses of cortisol restrain release of inflammatory cytokines by stimulating glucocorticoid receptors thereby bringing about cellular – and humoral immunity. As such glucocorticoids produce a series of systemic interactions that lead to a mainly anti-inflammatory effect, however low concentrations of glucocorticoids act permissively and under certain conditions they can aid in promoting inflammation (Hänsel et al., 2010). Indeed, a chronically activated HPA axis...
results in decreased anti-inflammatory feedback consequently inducing heightened inflammation. Psychosocial stress has been linked to increase in inflammatory biomarkers such as IL-6 and CRP (Rohleder et al., 2009), and in expression of pro-inflammatory genes (Miller et al., 2008). Subsequently it has been proposed that increases in circulating inflammatory levels induced by chronic stress are a consequence of changes in stress system activity brought about by an inadequate response to stress hormones that fundamentally allows for the disinhibition of pathways for inflammatory signaling (Strahler et al., 2014). Chronic psychological stress due to job stress, burnout, low socioeconomic status, childhood adversity and neglect, caregiving stress and loneliness has been found to correlate to higher levels of inflammatory markers (Mastorakos & Ilias, 2000). Increased expressions of inflammatory genes such as IL-6 have been found in postmortem brains of suicide victims (Hoyo-Becerra et al., 2013; Pandey et al., 2012; Tonelli et al., 2008). There exist an association between suicidal behavior, HPA axis dysregulation and inflammation (Melmel et al., 2016) and a relationship between pro-inflammatory cytokines and schizophrenia (Miller et al., 2011), bipolar disorder (Modabbernia et al., 2013) and MDD (Capuron & Castanon, 2016; Karlović, Serretti, Vrkić, Martinac & Marčinko, 2012). Chronic stress has moreover been known to cause neuroinflammation (Cheng et al., 2016; Sahin, Dursun, Cetin & Aricioglu, 2016) and glucocorticoid resistance in arthritis patients (David et al., 2008). Twin studies have reported that heightened inflammation is linked to higher levels of depressive symptoms over time and not the other way around. Inflammation could thus potentially cause depression and the association was found to be stronger in MZ than in DZ twins indicating that genetic confounding cannot explain the relationship (Huang et al., 2017).

In summary the relationship between the HPA axis and the immune system relates to their end products cortisol and cytokines. The bidirectional feedback loop between the two systems is responsible for cortisol’s ability to dysregulate the immune system resulting in systemic inflammation and likewise for cytokine ability to dysregulate the HPA axis and bring about mood symptoms.

There is reason to believe that the mood deterioration that follows the disrupted homeostasis might also be influence by the effect cytokines induce upon dopamine. Cytokines have frequently been reported as affecting the basal ganglia and dopamine to bring about depressive symptoms including motivation and motor activity (Felger, 2017; Caupron & Miller, 2011). The neurotransmitter has an established influence upon the inflammatory system and can be synthesized in neurons as well as in immune cells (Toth, Vecserynes, Zelles, Kadar & Nagy, 2012). Previous studies have reported that dopamine is a key neurotransmitter molecule maintaining multiple connections between the immune – and nervous system (Toth et al., 2012), as it can trigger changes in cytokine secretion (Toth et al., 2012).

**Positive psychological factors and biomarkers of immunity and the HPA axis**

The bidirectional relationship between the immune system and the HPA axis is however not exclusively related to pathology and negative affect. Evidence regarding the beneficial effect of positive affect, emotional well-being, life satisfaction and optimism has also been reported suggesting that the connection between the systems can be utilized for benefactor effects on physical and mental health. According to Pressman and Cohens (2005) stress-buffering model positive affect brings about positive health benefits through its ability to mitigate the pathogenic influence of stress. The relationship between positive affect and inflammation was recently investigated in a cross-sectional study. Positive affect levels were inversely related to perceived psychological stress levels. Higher levels of stress were
successfully buffered against by high positive affect thereby protecting against systemic inflammation (Blevins, Sagui & Bennet, 2017). Trait positive affect is further associated with lower production of cytokines, a similar relationship was not found for negative affect (Prather et al., 2007). Indeed lack of positive emotions appears highly relevant for systemic inflammation (Deverts et al., 2010). Even minor daily experiences can buffer against stress as daily positive events were found to act as a protective agent against systemic inflammation (Sin et al., 2014).

There is a growing body of evidence suggesting that there exists a direct pathway between health and positive affect, encompassing reduced psychobiological activation of neuroendocrine, autonomic, immune and inflammatory pathways. These beneficial health effects are independent of negative affect proposing that positive affect possess characteristic biological correlates (Dockray & Steptoe, 2010). Positive psychological factors are known to undo the physiological effects of stress upon the body (Sin et al., 2014), as well as reduce physiological reactivity to stressors (Aschbacher et al., 2012). Similarly individuals with a high positive emotional style are three times less likely to develop infection symptoms (Dockray & Steptoe, 2010). In a cross-sectional study employing a sample consisting of 8542 participants aged 32-86 the interaction between perceived stress and positive affect was tested in predicting mortality risk over a 10 year follow up period. The results showed that higher levels of positive affect were linked to lower mortality risk and withstood following adjustment for demographic factors and depressive symptoms, chronic disease and health behaviors (Okely et al., 2017). In a study by Stellar and coworkers (2015) 94 undergraduates were recruited, IL-6 levels and positive and negative affect schedule (PANAS) scores were obtained. The results showed that trait positive affect was negatively correlated with levels of IL-6 and this effect remained when controlling for BMI. These results were followed up with a second study in which 105 undergraduates left samples of IL-6, as well as PANAS and Dispositional Positive Emotions Scale (DPES) scores. The results demonstrated that dispositional joy, contentment, pride and awe each produced a negative correlation with levels of IL-6 (Stellar et al., 2015). Similar reports are elicited from a cross-sectional study featuring consecutive outpatients diagnosed with chronic heart failure in which the results show that positive affect was associated with reduced inflammation (Brouwers et al., 2012).

In regards to other positive psychological factors evidence have yielded mixed results with some reporting an association between life satisfaction and lower CRP (Hamer & Chida, 2011), whilst other studies have reported no such connection (Carpenter et al., 2012; Friedman & Ryff, 2012). Ironson, Banerjee, Fitch and Krause (2016) recruited 1979 participants from whom they obtained blood samples as well as measures of life satisfaction, positive affect (PANAS) as well as depression (CES-D). The study was cross-sectional in nature and the results showed that positive emotional well-being and life satisfaction were associated with lower CRP levels even after controlling for demographic variables and depression. The relationship between emotional well-being and lower CRP was partially mediated through health behaviors, this was however not the case for life satisfaction who remained significantly related to lower CRP levels even after controlling for health behavior. By this nature it follows that life satisfaction uniquely relates to CRP beyond the effect of positive affect (Ironson et al., 2016).

Research with clinical populations reveal a connection between optimistic versus pessimistic expectancies and clinical outcomes and as a result it has been proposed that optimistic mindset may predispose generally to a more robust immune function and consequently better health. HIV patients low on optimistic expectancies show lower survival rates and an earlier onset of AIDS than their more optimistic peers. A similar pattern was found with individuals that had undergone heart transplant in which optimistic expectancies
predicted better health six month post operation. Optimistic expectancies further appear to correlate with higher levels of certain immune cells. Likewise optimism has been found to moderate the negative impact of stress upon immunity (Segerstrom & Sephton, 2010). Brydon, Walker, Wawrzyniak, Chart and Steptoe (2009) report that in individuals injected with salmonella typhoid vaccine and exposed to stress, a strong positive relationship between optimism and antibody response could be found, proposing that stress attenuates the bodies antibody response to inflammatory stimulus in optimistic individuals and as such optimism may aid in promoting health. The results showed that in a stress condition there was an inverse relationship between IL-6 measure and optimism and that this relationship remained when controlling for age, BMI, baseline levels of IL-6 and depression (Brydon et al., 2009).

Segerstrom and Sephton (2010) conducted a study whose primary incentive was to examine the relationship between optimistic expectancy and in vivo immunity. The results revealed a connection between cell mediated immunity and expectancy in which increase in expectancy was followed by an increase in CMI. Contrariwise a decrease in expectancy correlated to a decrease in CMI. This relationship could be partially explained through positive affect exclusively. These findings suggest that the effects of positive affect upon health go beyond the absence of negative affect proposing that there is an equal importance for leading a happy life as it is to lack anxiety in terms of immunological health (Segerstrom & Sephton, 2010).

Majority of studies regarding the relationship between affectivity, stress and inflammation have been conducted in a Western cultural context. Little or none research has been conducted regarding how the relationship between negative affect and inflammation would translate to an Eastern culture. This is particularly interesting given the contrasting approach to negative emotion that can be found within Western and Eastern cultures. On account of this, Miyamoto and coworkers (2013) employed data from 1044 American and 382 Japanese individuals regarding self-assessment questionnaires measuring negative affect as well as serum IL-6 through blood samples. These data were also followed up with questionnaires regarding demographic variables, health behaviors and health status. The cross-sectional study showed that in agreement with previous findings there was an association between negative affect and elevated levels of IL-6 in Americans and these effects remained even after controlling for life style, health status, demographic variables and psychological factors. Similar effect could however not be found within the Japanese sample in which the association between negative affect and the pro-inflammatory cytokine was not evident. These results are taken as accentuating the role of cultural context as Eastern cultures are known to view negative emotions as far less problematic than Western cultures. Indeed within an Eastern cultural setting negative emotion are accepted and viewed as a necessary factor for self-improvement (Miyamoto et al., 2013). Positive psychological factors thus appear to target the bidirectional feedback loop between the HPA axis and the immune system, however, the aforementioned factors are all components of subjective well-being.

**Subjective well-being**

Well-being is a multidimensional construct according to contemporary research. Most prominently viewed through the perspective of *subjective well-being* the construct is thought to represent “happiness” and is composed of high positive affect, low negative affect, optimism and life satisfaction. Subjective well-being is a measure of the individual’s level of happiness and self-realization (Gale, Booth, Mottus, Kuh & Deary, 2013). Apart from its affective elements subjective well-being thus also encompasses cognitive elements such as optimism and life satisfaction (Davydov & Czabak-Garbacz, 2017). *Optimism* is defined as
persistent and positive thoughts, beliefs and forecasts about the future. Life satisfaction is yet another cognitive parameter of subjective well-being not representative of current mood but rather of a global evaluation of the individuals’ life (Mojon-Azzi & Sousa-Poza, 2011). Subjective well-being is proposed to decrease mortality from all causes including cardiovascular disease (Chida & Steptoe, 2008). Furthermore, individuals high in aspects of subjective well-being have higher resilience when facing stressors and stronger protective mechanisms against psychosocial disability following stress (Davydov & Czabak-Garbacz, 2017; Davydov et al., 2016; Davydov & Perlo, 2015; Davydov, Stewart et al., 2012). It has also been reported that life satisfaction can mitigate the relationship between socioeconomic status (SES) and physical health (Zilioli, Imami & Slatcher, 2015). Individuals high on aspects of subjective well-being are lower on Cortisol Awakening Response (CAR) as opposed to individuals low on trait well-being who have higher CAR (Mavioğlu & Duman, 2016). Subjective well-being is also linked to dopamine. Healthy individuals who were exposed to L-DOPA, an amino acid that is the precursor to dopamine, reported high levels of happiness following a small reward in a gambling task (Rutledge et al., 2014). Taken together, the evidence indicate that subjective well-being influences health and alters vulnerability and resilience to stress and psychopathology.

In summary then, the evidence point toward an existing relationship between the immune system and the HPA axis in the form of a bidirectional feedback loop. Stress and fundamentally an overly active HPA axis secrete cortisol that can stimulate an immune response in the absence of any tissue damage or pathogen. Likewise, when inflammation is present within the system inflammatory cytokines activate the HPA axis and initiate secretion of cortisol resulting in mood deterioration when the HPA axis becomes dysregulated. Cortisol level is a reliable marker of HPA axis activity and IL-6 and CRP levels within blood indicate systemic inflammation and are thus reliable biomarkers of immune response. When homeostasis between the HPA axis and immune system becomes disrupted by dysregulation of either system both systemic inflammation and mood symptoms will be present as has been reported by research with clinical populations. Positive psychological factors such as positive affect, optimism and life satisfaction have been found to independently correlate with lower inflammation and stress and might thereby be targeting the bidirectional feedback loop between the HPA axis and immune system. As subjective well-being encompasses all of these positive factors it may therefor impact upon the relationship between the HPA axis and immunity by reducing inflammatory and HPA axis biomarkers to a greater degree than positive affect independently, and aid in restoring homeostasis between the systems. As such subjective well-being could potentially reverse and relieve the symptomatology of the previously discussed medical and psychological pathologies and fundamentally aid in procuring better medical and psychological health outcomes. The relationship between affectivity and inflammation may however not be as unambiguous as previously believed as the influence of culture has been considered. Studies with Japanese samples fail to reproduce the relationship between negative affect and heightened inflammation. This is thought to indicate that culture might influence the connection between affectivity and health.

Objectives

The present study aims to test whether positive affect can lower inflammation and HPA axis activity, therefore individuals high on positive affect should be lower on inflammatory markers IL-6 and CRP and HPA biomarkers cortisol.

The study further intends to test whether subjective well-being corresponds to lower levels of inflammatory and HPA axis biomarkers. The study proposes that individuals with
higher levels of subjective well-being are lower on cortisol, IL-6 and CRP. As subjective well-being encompasses more positive psychological factors than positive affect the study also wants to test whether it is more effective in influencing the chosen biomarkers than positive affect. In order to strengthen the cause and effect pathway the study intends to employ a longitudinal design. As culture is proposed as a relevant factor the present study will also employ a Japanese sample in order to test whether effects of positive psychological factors on health reported in the Western world could be replicated in Japan.

The present study thus has three hypotheses. First, positive affect is a negative predictor for IL-6, CRP and cortisol. Second, subjective well-being is a negative predictor for IL-6, CRP and cortisol. Third, subjective well-being is a stronger negative predictor for IL-6, CRP and cortisol than positive affect.

**METHOD**

**Participants**

Respondents were a subset from the Midlife in Japan (MIDJA) study that was conducted as a parallel study to the Midlife in the united states (MIDUS) study. The initial study was conducted in 2008 and featured individuals recruited from the Tokyo metropolitan area who answered self-administered questionnaires. A subset of respondents were recruited for an extension study that aimed to collect biological data (N = 382, 214 women and 168 men with mean age of 54.24 years). The recruited participants visited a clinic in proximity to the University of Tokyo for data gathering and samples were frozen and shipped to the United States for analysis. A second wave of the study called; MIDJA II was instigated in 2012 that featured a follow up biomarker collection project (N = 328), and employed a substantial proportion of participants whom also completed the first wave of the biomarkers collection.

Participants were considered eligible for participation if they fulfilled the following criteria: (1) were featured in both the first and second wave of the biomarker project (2) had biomarker data and self-rating questionnaire data available (3) did not have a clinical physiological health diagnosis such as cancer, cardiovascular disease, arthritis, HIV, diabetes or had suffered a stroke.

The subsample that fulfilled all inclusion and exclusion criteria consisted of 174 participants, (60.3% female) between 36 and 85 years old (M = 57.23, SD = 13.1).

**Design**

The study had a within-subjects longitudinal design with two measurement points. All participants were tested on both points.

**Procedure**

The present study employed available published data from the The Midlife in Japan (MIDJA); Biomarkers project I and II. The original study collected baseline data in two stages, the initial phase consisted of survey data collection whilst biomarker collection occurred at a clinic and lastly participants also provided daily saliva samples and completed medical history questionnaires. The follow-up study followed the same procedure as the baseline collection. The original study collected measures of IL-6 and CRP levels through blood draw and blood assay. IL6 was measured using the Quantikine® high-sensitivity enzyme-linked immunosorbent assay (ELISA) kit #HS600B (R & D Systems, Minneapolis, MN). CRP was measured using kit #10446091 for the BNII nephelometer (Dade Behring).
Cortisol measures were obtained though saliva samples. Samples were collected by participants in their home setting following the visits to the clinic. Samples were taken at four different times a day, upon awakening, 30 minutes following awakening, at midday and at night each day for three consecutive days. Free cortisol concentration was determined using radioimmunoassay (catalog # 07-221106 from MP Biomedicals, Solon, OH). The assay kits have been employed as successful instruments for their respective biomarkers and reoccur often within research gauging biomarkers as well as in clinical conditions and should as should be instruments with good validity and reliability. The original study further obtained data regarding BMI, neuroticism, SES, perceived stress, age and sex. Finally the MIDJA: Biomarker project also obtained measures of subjective well-being through the Subjective well-being: Japanese comparison scale (α = .87). As well as measures of positive affect through the Positive affect scale (α = .93). In order to answer the hypotheses the present study thus employed data from the original study regarding the variables subjective well-being and positive affect to use as independent variables. As the study intended to use IL-6 and CRP as markers of inflammation, data regarding these variables was obtained from the original study. HPA axis activation was within the present study defined as cortisol levels and as such data regarding this variable was also taken from the original study. Finally the present study also employed data regarding BMI, neuroticism, SES, perceived stress, age and sex from the original study to use as control variables. The original study had collected data regarding the intended variables from participants at two different time points over a period of four years. Data from both of these collection points was used within the present study to enable a longitudinal approach.

### Statistical Analysis

The study’s hypotheses proposed that subjective well-being and positive affect would emerge as negative predictors of the chosen biomarkers. The study further employed a longitudinal design as participants were tested at two time points. This gives rise to hierarchical data in which the repeated measure is nested within participants. For these reasons it was concluded that the hypotheses would be best answered if a multilevel linear model was employed for analyzing the data.

Power was calculated post hoc to control if the study had featured a large enough sample and was computed with GLIMMMPSE for multilevel models. It was calculated that an α = .05 and a corresponding β = .95 in a within-subject repeated design with measures repeated at two time points and for a two level model would require a sample size of 54 participants.

Prior to conducting the multilevel linear model analysis outliers were identified and analyses were run including and excluding the outliers, as they did not alter the results they were kept. Data were initially skewed and produced a non-normal distribution, as a consequence thereof all non-normally distributed and skewed variables were log transformed. No variables were significantly correlated apart from CRP and IL-6 (r = .49). Cortisol was measured on three consecutive days across which the average was used in the analysis.

The multilevel data was identified as containing two levels, the first level units was the repeated measures and the second level units were the subjects and as such data produced a two-level hierarchical structure. When running the mixed effect models time point (1 and 2) was defined as the repeated measure and participants as subject variables, as the difference between measurements was constant a scaled identity covariance matrix was chosen for the repeated data. According to Field (2017) multilevel models allow for fixed and random effects, in which the fixed effects reflect that estimates are fixed across cases while random effects allows parameters to vary from case to case. Random effects are often random
intercepts and random slopes that represent a variance in intercepts and or slopes in the relationship between the dependent and independent variable across participants. For this reason it was decided to employ a mixed effects model that featured fixed as well as random effects in the form of random intercepts and random slopes. An unstructured covariance matrix was chosen for the random effects as this covariance structure is general and assumes covariance to be unpredictable (Field, 2017). Restricted maximum likelihood estimates were calculated. Every model was run in four stages in which the baseline model was the initial one. The fixed effect was then added in the second model, the random intercept in the third and the random slope in fourth. The final model thus included all fixed and random effects.

The first analysis set featured positive affect as the independent variable and IL-6, CRP and cortisol as dependent variables. The second set of analysis defined subjective well-being as the independent variable and IL-6, CRP and cortisol as dependent variables. The analyses was run with models for each dependent variable and a follow up model that also included control variables age, sex (women were coded with the higher value), BMI, neuroticism, SES and perceived stress. All statistical analyses were conducted with IBM SPSS 24.

**Ethical considerations**

As the present study did not collect data on its own but relied upon available data gathered through the MIDJA I and II biomarker project the present study did not have any ethical considerations. The original study employed informed consent upon data collection and informed its participants of their right to confidentiality, anonymity and to cessation of their participation should they wish to do so. Participants were also informed of the objectives of the study as well as how the data would later be used and distributed prior to signing the informed consent. The present study did not encounter any violation of research subject’s confidentiality neither intentional nor discovered inadvertently, nor did it violate the terms of distributing the data and as such did not breach the terms of the written consent.
RESULTS

Descriptive data for dependent variables IL-6, CRP and cortisol and independent variables positive affect and subjective well-being as well as control variables are presented in Table 1.

Table 1. Means and standard deviations of both outcome, independent and control variables.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Time point 1</th>
<th></th>
<th></th>
<th>Time point 2</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Std. Deviation</td>
<td>Mean</td>
<td>Std. Deviation</td>
<td></td>
</tr>
<tr>
<td>Positive Affect</td>
<td>3.22</td>
<td>0.73</td>
<td></td>
<td>3.25</td>
<td>0.68</td>
</tr>
<tr>
<td>IL-6 (pg/ml)</td>
<td>1.30</td>
<td>1.30</td>
<td></td>
<td>1.33</td>
<td>1.32</td>
</tr>
<tr>
<td>Subjective Well-Being</td>
<td>2.92</td>
<td>0.48</td>
<td></td>
<td>2.86</td>
<td>0.53</td>
</tr>
<tr>
<td>CRP (ug/ml)*</td>
<td>0.76</td>
<td>2.00</td>
<td></td>
<td>0.91</td>
<td>2.35</td>
</tr>
<tr>
<td>Cortisol (nmol/L)*</td>
<td>7.05</td>
<td>2.40</td>
<td></td>
<td>11.42</td>
<td>3.96</td>
</tr>
<tr>
<td>Neuroticism</td>
<td>2.11</td>
<td>0.56</td>
<td></td>
<td>2.07</td>
<td>0.52</td>
</tr>
<tr>
<td>BMI</td>
<td>22.37</td>
<td>3.01</td>
<td></td>
<td>22.50</td>
<td>3.15</td>
</tr>
<tr>
<td>Age</td>
<td>56.03</td>
<td>13.46</td>
<td></td>
<td>57.23</td>
<td>12.98</td>
</tr>
<tr>
<td>PSS</td>
<td>26.43</td>
<td>5.50</td>
<td></td>
<td>26.02</td>
<td>5.90</td>
</tr>
</tbody>
</table>

Note. *Statistically Significant at p < .05 level between measurement points in a paired samples t-test, data values are shown following log transformation. Abbreviations; IL-6 (Interleukin-6) CRP (C-reactive protein), BMI (body mass index) and PSS (perceived stress scale).

In order to test the study’s hypotheses, linear mixed effects models were run to examine the effect of the independent variable on the dependent variables across the two measurement points across a four year period.

Positive affect and inflammatory biomarkers

It was predicted that positive affect would be a negative predictor for biomarkers IL-6 and CRP. In order to test the hypothesis mixed effects models were run that featured IL-6 as the dependent variable and positive affect as independent variable. The final mixed model for the relationship between positive affect and IL-6 had no significant variance in intercepts across subjects (\(\text{var}(u_{0j}) = 56.13, \chi^2(1) = 0.67, p > .05\)), slopes did not vary across subjects (\(\text{var}(u_{ij}) = 2.24, \chi^2(1) = 0.04, p > .05\)) and there was no significant covariance between slopes and intercepts (\(\text{cov}(u_{0j}, u_{ij}) = -10.06, \chi^2(2) = 2.68, p > .05\)). Positive affect did not produce a significant effect upon IL-6 and as such did not emerge as a relevant predictor for IL-6 (\(F(1, 109) = 0.21, p > .05, d = 0.03, b\)-estimates are presented in Table 2).

A similar pattern was found for the relationship between positive affect and CRP. The hypothesis predicted that positive affect would be a negative predictor for CRP. To test this assumption mixed effects model were run that featured CRP as the dependent variable and positive affect as the independent variable. The final model had no significant variance in intercepts across subjects (\(\text{var}(u_{0j}) = 56.13, \chi^2(1) = 1.14, p > .05\)), in variance in slopes across subjects (\(\text{var}(u_{ij}) = 0.03, \chi^2(1) = 1.04, p > .05\)), and no covariance between slopes and intercepts, (\(\text{cov}(u_{0j}, u_{ij}) = -0.11, \chi^2(2) = 1.32, p > .05\)). Positive affect was not a significant negative predictor of CRP (\(F(1, 110) = 0.22, p > .05, d = 0.37, b\)-estimates are presented in Table 2). Positive affect did thus not correspond to lower levels of inflammatory markers IL-6 and CRP as the hypothesis had predicted.
Positive affect and HPA axis biomarkers

The study proposed that positive affect would be a negative predictor for cortisol. To test this hypothesis mixed effects models were employed with cortisol as dependent variable and positive affect as independent variable. The final model had no significant variance in intercepts across subjects (var(\(u_0\)) = 7.58, \(\chi^2(1) = 14.92, p > .05\)), in variance in slopes across subjects (var(\(u_1\)) = 30.47, \(\chi^2(1) = 17.49, p > .05\)), and no covariance between slopes and intercepts (cov(\(u_0\), \(u_1\)) = -15.06, \(\chi^2(2) = 122.22, p > .05\)). Positive affect was not a significant negative predictor of cortisol (\(F(1, 168) = 0.99, p > .05, d = 0.13\). b-estimates are presented in Table 2). The hypothesis of positive affect corresponding to lower levels of cortisol could thus not be supported.

Subjective well-being and inflammatory biomarkers

The study also proposed that subjective well-being would be a negative predictor for IL-6 and CRP. To test this hypothesis mixed effects models were run with IL-6 as dependent variable and subjective well-being as the independent variable. The final model exhibited a significant variance in intercepts across subjects (var(\(u_0\)) = 26.33, \(\chi^2(1) = 21.30, p < .05\)), the slopes varied across subjects (var(\(u_1\)) = 19.27, \(\chi^2(1) = 11.64 p < .05\)) and slopes and intercepts significantly negatively covaried (cov(\(u_0\), \(u_1\)) = -71.28, \(\chi^2(2) = 58.66, p < .05\)). Subjective well-being negatively and significantly predicted levels of IL-6 (\(F(1, 172) = 4.42 p > .05, d = 0.50\), b-estimates are presented in Table 2), and the effect remained even after perceived stress, age, sex, SES and neuroticism were included in the model. This indicates that higher levels of subjective well-being correspond to lower levels of IL-6, the results thus lend support to the hypothesis.

With regards to CRP, mixed effects models were run with CRP as the dependent variable and subjective well-being as the independent variable. The final model had no significant variance in intercepts across subjects, (var(\(u_0\)) = 265.93, \(\chi^2(1) = 19.88, p > .05\)), the slopes did not vary across subjects (var(\(u_1\)) = 20.24, \(\chi^2(1) = 23.30, p > .05\)), nor did the slopes and intercepts covary (cov(\(u_0\), \(u_1\)) = -73.30, \(\chi^2(2) = 31.98, p > .05\)). The analysis found no support for the hypothesis of subjective well-being as a negative significant predictor of CRP (\(F(1, 172) = 0.29, p > .05, d = 0.05\), b-estimates are presented in Table 2). As such higher subjective well-being relates to lower levels of IL-6 but not lower levels of CRP.

Subjective well-being and HPA axis biomarkers

The study also hypothesized that higher levels of subjective well-being would predict lower levels of cortisol. To test this assumption cortisol was defined as the dependent variable and subjective well-being as the independent variable in mixed effects models. The final model showed significant variance within intercepts across subjects (var(\(u_0\)) = 10.76, \(\chi^2(1) = 21.45, p < .05\)), slopes also varied across subjects, (var(\(u_1\)) = 45.09, \(\chi^2(1) = 17.47 p < .05\)) and slopes and intercepts significantly covaried (cov(\(u_0\), \(u_1\)) = -13.07, \(\chi^2(2) = 25.03, p < .05\)). Subjective well-being was as a significant negative predictor of cortisol (\(F(1, 171) = 5.01, p < .05, d = 0.62\), b-estimates are presented in Table 2) and this effect remained when SES, sex, age, BMI, perceived stress and neuroticism were added to the model. As such the results are in favor of the hypothesis as higher levels of subjective well-being relate to lower levels of cortisol.
**Table 2. b-estimates for predictors and control variables.**

<table>
<thead>
<tr>
<th></th>
<th>$b$</th>
<th>SE $b$</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>IL-6 (pg/ml)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive Affect</td>
<td>-0.23</td>
<td>0.46</td>
<td>-1.12 – 0.69</td>
</tr>
<tr>
<td>Subjective well-being</td>
<td>-0.31*</td>
<td>0.15</td>
<td>-0.59 – 0.12</td>
</tr>
<tr>
<td>Sex</td>
<td>-0.56</td>
<td>1.25</td>
<td>-3.69 – 2.40</td>
</tr>
<tr>
<td>Age</td>
<td>0.33</td>
<td>0.50</td>
<td>-0.66 – 1.38</td>
</tr>
<tr>
<td>SES</td>
<td>0.40</td>
<td>1.79</td>
<td>-1.25 – 1.82</td>
</tr>
<tr>
<td>BMI</td>
<td>0.24</td>
<td>0.29</td>
<td>-0.34 – 0.83</td>
</tr>
<tr>
<td>PSS</td>
<td>0.11</td>
<td>0.14</td>
<td>-0.39 – 0.15</td>
</tr>
<tr>
<td>Neuroticism</td>
<td>0.78</td>
<td>0.69</td>
<td>-0.61 – 2.17</td>
</tr>
<tr>
<td><strong>CRP (ug/mL)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive affect</td>
<td>-0.06</td>
<td>0.08</td>
<td>-0.23 – 0.11</td>
</tr>
<tr>
<td>Subjective well-being</td>
<td>-0.14</td>
<td>0.17</td>
<td>-0.24 – 0.0</td>
</tr>
<tr>
<td>Sex</td>
<td>0.26</td>
<td>2.23</td>
<td>-4.45 – 4.99</td>
</tr>
<tr>
<td>Age</td>
<td>0.01</td>
<td>0.09</td>
<td>-0.17 – 0.19</td>
</tr>
<tr>
<td>SES</td>
<td>0.74</td>
<td>1.21</td>
<td>-1.77 – 3.26</td>
</tr>
<tr>
<td>BMI</td>
<td>0.09</td>
<td>0.05</td>
<td>-0.01 – 0.20</td>
</tr>
<tr>
<td>PSS</td>
<td>0.01</td>
<td>0.02</td>
<td>-0.03 – 0.05</td>
</tr>
<tr>
<td>Neuroticism</td>
<td>0.23</td>
<td>0.12</td>
<td>-0.48 – 0.11</td>
</tr>
<tr>
<td><strong>Cortisol (nmol/L)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive affect</td>
<td>-0.52</td>
<td>0.55</td>
<td>-0.53 – 1.62</td>
</tr>
<tr>
<td>Subjective well-being</td>
<td>-0.37*</td>
<td>1.66</td>
<td>-7.00 – 0.4</td>
</tr>
<tr>
<td>Sex</td>
<td>0.67</td>
<td>1.12</td>
<td>-0.92 – 1.05</td>
</tr>
<tr>
<td>Age</td>
<td>0.16</td>
<td>0.86</td>
<td>-0.53 – 0.56</td>
</tr>
<tr>
<td>SES</td>
<td>0.72</td>
<td>1.17</td>
<td>-0.85 – 0.71</td>
</tr>
<tr>
<td>BMI</td>
<td>0.21</td>
<td>0.51</td>
<td>-0.67 – 0.38</td>
</tr>
<tr>
<td>PSS</td>
<td>0.10</td>
<td>0.23</td>
<td>-0.35 – 0.57</td>
</tr>
<tr>
<td>Neuroticism</td>
<td>0.64</td>
<td>1.02</td>
<td>-0.40 – 0.75</td>
</tr>
</tbody>
</table>

Note. *Significant at $p < .05$. Outcome values are shown following log transformation. Abbreviations; IL-6 (Interleukin-6) CRP (C-reactive protein), SES (socioeconomic status), BMI (body mass index) and PSS (perceived stress scale).

**DISCUSSION**

The present study proposed that positive affect would predict levels of inflammatory biomarkers IL-6 and CRP and HPA axis biomarkers cortisol. The study also suggested that subjective well-being would predict IL-6, CRP and cortisol. Finally the study proposed that subjective well-being would be a stronger negative predictor for all biomarkers than positive affect. The analysis failed to find any support for the first hypothesis as positive affect was not associated with lower IL-6, CRP or cortisol. These findings are incongruent with results from previous research that have frequently reported positive affect as sharing an inverse relationship to levels of inflammatory cytokine IL-6 and acute phase reactant CRP. Previous research has demonstrated that positive affect is a reliable health predictor in healthy as well as clinical patients (Blevins et al., 2017; Okely et al., 2017; Sin et al., 2014; Brouwers et al., 2012; Deverts et al., 2010; Dockray & Steptoe, 2010; Prather et al., 2007), and as such the results procured here are diverging from previous studies. Explanatory potential may be
derived from the fact that this is the first study to examine the relationship between positive affect and inflammation within an Eastern cultural context as previous research has focused exclusively upon Western countries. This is not the first study that has been unsuccessful in replicating findings from Western countries in Japan as a previous study failed to reproduce the association between negative affect and elevated inflammation within a Japanese sample (Miyamoto et al., 2013). Likewise a recent study could not find any support for the association between positive affect and health biomarkers HDL (high-density lipoprotein) and DHEA-S (dehydroepiandrosterone-sulfate) within a Japanese sample but successfully established the relationship in an American sample (Yoo, Miyamoto & Ryff, 2016).

Western cultures have traditionally placed an emphasis on the pursuit of positive emotions and construed negative emotions as an adverse life impact that inhibits the individuals’ ability to yield control over her life. In contrast, Eastern cultures are characterized by a greater amount of dialectical thinking in which life is considered as being defined by opposites. It follows that within an Eastern cultural context happiness and unhappiness are regarded as co-existing and complementary to one another. Negative emotions are recognized and considered as a source of motivation for self-improvement. Japanese culture considers negative affectivity as intrinsic rather than something to be avoided and there is reason to believe that the negative health outcomes reported in Western cultures would be less evident in Japan (Miyamoto et al., 2013). There exists at least some support for this within the literature as balance between moderate amounts of negative and positive emotions are linked to better health outcomes in Japan but not in the United States (Miyamoto & Ruff, 2011). According to Uchida and Kitayama (2009) ideal positive affect within Japanese cultures is subdued and as such low in intensity and arousal. Low arousal emotions such as content and calmness are considered desirable for gaining happiness. Following this line of reasoning negative emotions will not be as psychologically costly in Japan as opposed to a Western country such as the United States. It should also be highlighted that the Japanese mindset might have influenced the present results. As moderate amounts of negative but also positive affect are desired, the influence of high positive affect that is reported to bring about positive health outcomes in Western cultures might not be made evident in this sample. In point of fact, simply a lack of negative affect is not sufficient to generate positive health outcomes, but higher levels of positive affect appear as a critical requirement to enjoy any benefits on physiological health (Segerstrom & Sephton, 2010). Western cultures that aspire an inclination towards high positive affect might as such generate a cultural mindset that welcomes and cultivates positive emotions and might, in turn, yield the results reported in previous studies. As moderate affectivity is considered desirable there remains a possibility that low variance within subjects might have reduced the effect of positive affect within the data as the sample may have been too small to successfully pick up on such an effect. For this reason future research with Japanese participants should strive to use a larger sample when examining affectivity dimensions.

The second hypothesis of the present study was that subjective well-being would influence inflammatory and HPA markers. This hypothesis was supported by the data, as subjective well-being was a significant negative predictor for IL-6 and cortisol albeit not for CRP. This suggests that subjective well-being is a better predictor for lowered levels of acute inflammatory processes than for chronic instances. CRP has a short life-span, circulating levels within blood are believed to reflect that chronic inflammation is present within the system (Measelle et al., 2016). The synthesis and regulation of CRP is driven by inflammatory cytokines such as IL-6, as such when CRP levels are high circulating IL-6 will also be present as it initiates the acute inflammatory response (Halaris, 2016). Subjective well-being it would appear is thus not sufficient to constrain inflammation that has researched
the chronic stage. As subjective well-being failed to produce a relevant effect on CRP, this is contradictory to previous studies that successfully established a link between life-satisfaction and emotional well-being and lowered CRP levels. The findings reported here are more inclined towards results from Carpenter et al., (2012) and Friedman and Ryff, (2012) in which life satisfaction was found to be irrelevant for CRP levels. The present results may also relate to the fact that previous studies have employed mainly cross-sectional designs whilst the present study featured a longitudinal approach. It should also be stressed that in regards to the relationship between affectivity and health, IL-6 has been relied upon as a biomarkers of inflammation to a greater extent than CRP. The great majority of work that has been conducted has tested IL-6 exclusively and this might have influenced the literature as IL-6 is initiated early on in the inflammatory process as opposed to CRP that reflects chronic inflammation to a greater degree.

The results regarding subjective well-being as a relevant predictor for lower IL-6 and cortisol levels are congruent with research regarding the effects of optimism (Segerstrom & Sephton, 2010; Brydon et al., 2009), life satisfaction (Ironson et al., 2016; Hamer & Chida, 2010) and positive affect (Blevins et al., 2017; Okely et al., 2017; Sin et al., 2014; Brouwers et al., 2012; Deverts et al., 2010; Dockray & Steptoe, 2010; Prather et al., 2007) on the relationship between the HPA axis and the immune system. Moreover individuals high on life satisfaction are lower on CAR (Mavioglu & Duman, 2016), and are known to possess higher levels of resilience towards stress (Zilioli et al., 2015). Alternatively the results can be considered as indicating that lower levels of inflammation and HPA axis activity in the body gives rise to greater subjective well-being and as such relate to the effect of dopamine. As inhibited dopamine synthesis is related to sickness behavior and anhedonia it could be argued that those individuals who have low levels of inflammatory markers and consequently inhibited systemic inflammation would have greater levels of dopamine and therefore greater subjective well-being as these two factors are correlated (Rutledge et al., 2014). Studies report that induced inflammatory state in healthy or medically ill subjects will increase levels of inflammatory cytokines and thereby increase the prevalence of mood symptoms (Rosenblat et al., 2014). Inflammatory cytokines can stimulate cortisol secretion from adrenocortical cells and can during chronic instances dysregulate the HPA axis and bring about sickness behavior (Rosenblat et al., 2014). By this nature it then follows that those who are low on biomarkers of inflammation will not suffer mood related symptoms and enjoy greater subjective well-being. Dopamine and its downstream signaling further possesses an anti-inflammatory function and control systemic inflammation (Shao et al., 2013). Dopamine can regulate inflammation by inhibiting NLRP3 (Yan et al., 2015). The NLRP3 inflammasome is assembled in response to endogenous danger signals as well as pathogens (Davis et al., 2011; Martinon et al., 2009), and is responsible for the maturation and the release of many pro-inflammatory cytokines and is therefore crucial in initiating inflammation (Lamkanfi and Dixit, 2012; Schroder and Tschopp, 2010). Dopamine successfully inhibits NLPR3 inflammasome activation through dopamine D1 receptor (DRD1) and dopamine signaling inhibits inflammasome-dependant depression such as neuroinflammation (Yan et al., 2015). As subjective well-being and dopamine are linked individuals high on subjective well-being could also be reaping the benefits of the neurotransmitters anti-inflammatory effect.

From an ethical position it should nonetheless be accentuated that these findings should not be taken as indicative that dysregulation in the HPA axis and the immune system is exclusively related to low subjective well-being. The severity of medical and psychopathological ailments should not be reduced and diminished to reflect low subjective well-being, indeed these pathologies are complex and variance in symptoms will occur
between individuals. Likewise one should not accept accounts of sickness behaviors as reflective of systemic inflammation in the absence of well-being or vice versa, this area of study is in its infancy and should be treated as such.

The third hypothesis predicted that subjective well-being would be a stronger negative predictor for all biomarkers than positive affect. As positive affect was not a significant predictor for any biomarker and subjective well-being successfully predicted IL-6 and cortisol, albeit not CRP, this hypothesis has been supported by the results. As positive affect is a key component of subjective well-being this gives rise to the question as to why it could not independently invoke an influence upon either acute or chronic inflammation nor HPA axis activity. In contrast it would appear that the cognitive components within the construct are accountable for the reported effect. Again, this could relate to the sample chosen for the study, due to a neutral outlook on negative feelings positivity is made yet another fading aspect of life and will not bring about the same feelings of satisfaction as they are given within a Western context. It is possible that the cognitive appraisal of one’s feelings and the invoked meaning bestowed upon these feelings might influence Westerners more than Easterners. Granting all this, the strongest explanatory mechanism underlying this discrepancy may relate to affectivity as a temporary and changing aspect of the individuals life, whilst evaluation of optimism and life satisfaction encompass a measure of personal tendency that reflect a more permanent assessment of oneself. These findings help strengthen the proposition that life satisfaction and optimism uniquely relate to levels of inflammatory markers beyond the impact of positive affect (Ironson et al., 2016). Subjective well-being is further related to dopamine, and as such high levels of subjective well-being could initiate the proposed anti-inflammatory potential of the neurotransmitter (Yan et al., 2015). In light of the finding that positive affect failed to account for lower inflammatory levels it would appear that in Japanese individuals, positive affect is not sufficient on its own but requires cognitive components such as life satisfaction and optimism to induce positive health effects.

Hereinafter these results need to be seen within the wider context pertaining to the bidirectional nature of the relationship between subjective well-being, inflammatory markers and HPA markers. Individuals undergoing medical treatment or suffering from chronic ailments may suffer a decrease in life quality due to the impact of circulating inflammatory cytokines upon the HPA axis and ultimately upon mood consequently increasing the risk for anhedonia and sickness behavior (Rosenblat et al., 2014). As such these results can caution for the relevance of counseling and psychological support for individuals who suffer from medical conditions. The reported relationship between medical ailment and suicide proves that many of our common disease may decrease psychological well-being and impose a detrimental effect on the individual (Brundin and Grit, 2016; Lund-Sorensen et al., 2016; Goodwin, 2012). The present results propose that when elevating subjective well-being these detrimental health effects may be avoided as it correlates to lower inflammatory cytokines who are implemented in sickness induced suicide behavior and mood deterioration as well as in HPA axis dysregulation, another known cause for suicidal behavior (Melhelm et al., 2017). As IL-6 dysregulates the HPA axis it is an inflammatory cytokine that could thus potentially be lowered through efforts to elevate subjective well-being and fundamentally decrease the risk for sickness behavior and psychopathology. Implementing positive psychotherapy and other treatment related parameters for clinical populations could possibly aid in regulating and maintaining symptoms of coronary heart disease, stroke (Yang et al., 2015), asthma and allergies (Goodwin, 2012), cardiovascular disease (Hänsel et al., 2010), schizophrenia (Miller et al., 2011), bipolar disease (Modabbernia et al., 2013), depression (Melhelm et al., 2017), anxiety (O’Donovan et al., 2010) and arthritis and rheumatology (Fuller-Thompson et al., 2016; Mastorakos & Ilias, 2000) among others. Likewise decreasing inflammation through
increase in subjective well-being could potentially restrain the tissue damage that is known to develop over time in systemic inflammation and give rise to pathology (Strahler et al., 2014). Increase in subjective well-being might decrease the inflammatory signaling that is known to impair neuronal health, dysregulate neurotransmitters and alter neural activity (Miller et al., 2013). Subjective well-being is not an adamant factor; it can be altered in a positive direction through means such as mindfulness training and psychotherapy (Zilioli et al., 2015) as well as cognitive restructuring (Ironson et al., 2016). Subjective well-being is further found to be impacted through positive writing, gratitude, life coaching, the happiness program, mindfulness, behavioral activation, pleasant activities, social activation and positive psychotherapy (Ironson et al., 2016). These factors could be implemented in clinical settings with the aspiration to generate positive health outcomes. Due to the bidirectional nature of this relationship it should be stated that maintaining a high level of subjective well-being could be particularly beneficial for physiological as well as psychological health. Indeed, positive psychological factors can undo the physiological effects of stress upon the body and reduce physiological reactivity to stressors (Sin et al., 2014; Aschbacher et al., 2012).

Fundamentally it cannot be overlooked that the present results may reflect an acute reaction due to the clinical test setting as heightened cortisol will suppress inflammatory cytokines, IL-6 particularly (Hänsel et al., 2010). As participants were faced with the blood-draw and medical evaluation they may have experienced acute stress due to feelings of unease, as such cortisol would increase and suppress IL-6. On the other hand cortisol could thus have declined until salivary cortisol samples were obtained in the comfort of the participants’ home. The results are however compatible with reports from previous studies regarding the suppressing influence of positive psychological factors upon acute stress and inflammation (Blevins et al., 2017, Rosenblat et al., 2014). Individuals high on subjective well-being are further also reported as having healthier cortisol circadian profiles than those who have low subjective well-being (Mavioglu & Duman, 2016). The usage of salivary cortisol measures could be argued as a potential methodological weakness within the study, and the study could potentially have been strengthened had urine cortisol levels been obtained instead of saliva cortisol. The current study featured HPA axis biomarkers that were collected by the participants themselves in the three days following the clinical visit but could have been obtained during the visit to the clinic with other samples. The method implemented within the study did however allow for obtaining an average over time that was regarded as a stronger measure of the general cortisol level that would not have been made possible had the sample only been gathered during the visit to the clinic. It should also be highlighted that the instruments that were used for gauging IL-6, CRP and cortisol have long standing clinical and research use and are successfully employed when measuring the specified biomarkers. These assay kits should as such have high validity and successfully capture true values of biomarkers. In terms of reliability, different reactions and responses can occur in the body in a short period of time due to different factors and it could perhaps be argued that this poses some difficulty for testing the overall consistency of the measures and thereby establish reliability. As the assay kits have been employed for a long duration of time and repeatedly tested they should however reflect a consistent measure in cases when no profound difference in immunological or endocrinological response has occurred.

In summary, the study has showed that higher levels of subjective well-being correspond with lower inflammation and HPA axis activation thereby proposing that subjective well-being could be implemented within clinical settings to obtain better mental and medical health outcomes. As subjective well-being was associated with lower inflammation and HPA axis activation it may be efficient enough to target the bidirectional feedback loop that obtains homeostasis between these two systems. Subjective well-being
could thus potentially relieve the symptoms and reverse the negative health effects that arise when homeostasis between the HPA axis and the immune system is disrupted. The importance of cultural background in regards to research dealing with the connection between psychology and physiology has also been made evident. The influence of positive affect often reported could not be replicated within a Japanese sample, however the study was successful in finding an association between subjective well-being and inflammatory cytokines and cortisol proposing that subjective well-being might be an even stronger predictor for positive health outcomes than positive affect. Future research should take into account that cultural differences may complicate generalizing findings and fundamentally implementing theoretical knowledge in practical settings. It should further strive to examine the components of the construct of subjective well-being in depth with the aim of predicting health and utilize longitudinal studies with many touchpoints in doing so. Lastly neurotransmitter dopamine is implemented as an important aspect of the relationship between the immune system and HPA axis, it is elevated in individuals with high subjective well-being and could thus potentially mediate the relationship. Therefore more research must be conducted taking into account the effect of dopamine, as this neurotransmitter is found to be a potential factor behind anhedonia in sickness behavior and bidirectionally an anti-inflammatory at elevated levels with potential for implementation in clinical settings.

Limitations

There are a number of limitations that are particularly relevant for the present study. First the measure of subjective well-being was obtained through a culturally sensitive instrument and should as such be adequate for measuring the construct within the intended population. The same cannot be highlighted for positive affect in which a measurement not culturally sensitive was employed. These results are thus comparable to Western studies but may not successfully incorporate a view upon positive affect that is relevant for the culture in which it was employed. This might constitute a problem for construct validity as the construct of positive affect measured by the instrument may not have reflected positive affect dimensions relevant to the Japanese society which appears to rely upon a higher degree of low arousal emotions. Self-assessment questionnaires further cannot bypass the individuals’ inclination to report scores in high congruence with the cultural norm. The accuracy and thereby the sufficiency of the self-assessment questionnaires relies heavily upon whether participants truly understood the question and answered them in a truthful way. Fixed choice question are quantifiable but lack flexibility and can thereby result in the participant leaving an answer they do not fully identify with. The questionnaire employed in the present study therefore featured likert-type scales in order to eliminate this effect. These potential threats should further been reduced through the implementation of repeated measures within the longitudinal design. The MIDJA and MIDUS samples are moreover known to have a higher educational level than the majority population (Miyamoto et al., 2013), and as such these findings could be reflective of a relationship between health and subjective well-being that potentially would not arise within individuals of lower SES. The results may therefore be reflective of higher subjective well-being and health due to life circumstances. Albeit the participants included did indeed have lower BMI and a higher educational level than most members of the Japanese society the study controlled for BMI and SES and found no significant influence on the results. Finally, as the study was conducted with a Japanese sample and the cultural background of the individuals favored a dialectic and moderate relationship to affectivity the results may not transfer to samples from other cultures. This limitation was accepted within the study as it hoped to examine whether results from the Western world could be replicated in Japan and thereby stress the importance of considering
culture. The study could moreover have benefitted from incorporating further measures, the study encompassed two touch points during a 4 year period and it could be argued that obtaining data from multiple time points and for a longer duration of time would have yielded more insight into the relationship between the variables. One should also not bypass that certain differences may have been brought about by genetic predisposition as individuals that are carriers of the long allele 5-HTTLPR gene are found to have higher values of subjective well-being than those who carry the short allele (Mavioglu & Duman, 2016). Albeit genetic predisposition is difficult to disregard it is unlikely that the findings described here relate exclusively to genetic differences as twin studies have reported that the relationship between mood and inflammation is not genetically confounded (Huang et al., 2017).

**Conclusion**

The study found no support for the previously reported effect of positive affect upon inflammation or HPA axis activity. Subjective well-being, on the contrary, emerged as a negative predictor of inflammation and HPA axis activation suggesting that subjective well-being is associated with lower systemic inflammation and stress-induced effects on the body. Therefore the study proposes that cognitive components of subjective well-being such as life satisfaction and optimism may uniquely relate to health in a relationship that goes beyond positive affect. The study also found that findings frequently reported from the Western world in regards to the beneficial health effects of positive affect could not be replicated in Japan and as such proposes that the impact of culture might be worth taking into account in psychoneuroimmunological research.
REFERENCES


