Epigenetics and endocrinology: new frontiers in motivational science

Epigenetics and Endocrinology: New Frontiers in Motivational Science

By

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Abstract

This thesis explored the role of motivational processes in improved human functioning. There is a large body of evidence supporting the idea that higher quality motivation can have important implications for health and well-being via improved behavioural engagement. However, what is less well understood is whether there is a direct effect of motivation on physiological health beyond that which is explained by engagement with the activity itself. The aim of this thesis was to explore associations between motivational processes and biological markers implicated with improved physical health and human functioning.

The programme of research began by systematically reviewing multidimensional theories of motivation and physiological responses associated with activation of the endocrine system. Across several conceptually similar theories of motivation, it was evidenced that higher quality motivation was associated with an attenuated cortisol response in a variety of environments eliciting social-evaluative threat. Furthermore, the needs for power and affiliation were associated with lower and higher levels of salivary secretory immunoglobulin A, respectively. Based on the limitations identified in the systematic review, two subsequent chapters further explored the role of high quality motivation in reducing the cortisol response. Using an experimental design grounded in self-determination theory, Chapter Three explored the effect that manipulating autonomous and controlled motivation had on participants' cortisol responses, while concurrently measuring self-control exertion. A repeated measures ANCOVA revealed a significant quadratic interaction (F (1, 32) = 5.40, p =.027, η_p^2 = .14). Participants in the controlled regulation condition experienced an increased, with autonomy supportive experiencing a decreased, cortisol response. There was also evidence of motivational effects on self-control performance, although the results contradicted the hypothesis. A mixed ANCOVA revealed participants in the controlled regulation condition recorded greater wall sit performance in the first and second wall sits compared with the autonomy-supportive condition (F (1,36) = 4.40, p = .043, η_p^2 = .11).

This concept was replicated and extended in Chapter Four. Although the cortisol pattern was not replicated, nonetheless a repeated measures ANOVA revealed a significant linear within subject contrast (F (2, 57) = 4.45, p = .016, η_p^2 = .14), with a significant linear decline in cortisol observed in the control condition. Furthermore, there was support for the idea of high-quality motivation improving self-control performance. A one-way ANOVA revealed a self-control

performance difference between experimental conditions (F (67,2) = 2.88, p = .063, η_p^2 = .08), that was driven by autonomy-supportive participants outperforming those exposed to controlled regulation (p = .055). As predicted, this performance difference did not persist to the second self-control task, the plank (F (64,4) = 0.41, p = .664, η_p^2 = .03).

In the final empirical chapter, the focus shifted from proximal to stable long-term markers of health by exploring a novel area for self-determination theory – epigenetics - specifically, the DNA methylation of the *TNF* gene associated with expression of the proinflammatory cytokine TNFα. The results further supported the idea that high quality motivation is most important for health-related behaviour and outcomes. Health-related autonomous motivation was weakly but positively associated with *TNF* methylation (β =.18, p =.08); however, health-related introjected regulation was not associated with *TNF* methylation (β =.13, p =.23). In a subsequent step, path analysis was used to explore whether this relationship persisted when accounting for healthy behaviour, however direct effects of autonomous motivation on *TNF* methylation did not persist when these indirect effects were included (β =.09, p =.43).

Overall, this thesis found support for the value of high-quality motivational processes at the epigenetic and endocrine level. Autonomous motivation was indirectly associated with DNA methylation of *TNF*, via engagement in healthy behaviour. Furthermore, there were several examples of evidence suggesting that high-quality motivation was associated with attenuated cortisol profiles, and conversely, lower quality motivation was associated with an increased cortisol response. There was also evidence that autonomous motivation was implicated in improved self-control performance, but this was not consistently observed. By extending research into epigenetics and endocrinology, this thesis supports the idea that high-quality motivation has important implications for improved health and well-being

List of publications arising from this thesis

Conference proceedings:

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Chapter One

Literature Review

General Introduction

In a historical sense, motivation was viewed as a unidimensional psycho-physiological process concerned with the direction and energization of goal-directed behaviour (Elliott, 2006). Classical behaviourist theories conceptualise motivation as a drive to satisfy basic physiological needs, such as hunger, thirst and sexual appetite (Hull, 1943), with less emphasis on psychological needs (e.g., Maslow, 1943). However, the post-cognitive revolution prompted motivational researchers to explore cognitive-behavioural theories concerned with the strength of beliefs in predicting behavioural outcomes (Bandura, 1977). For Bandura, motivation (viz. self-efficacy) is a key mechanism for explaining how individuals engage in goal-directed behaviour. In other words, a greater level of motivation is associated with greater behavioural engagement, and therefore, a greater likelihood of achieving optimal outcomes. Building on this work, motivational scientists began not only to consider *how* individuals are psychologically motivated to engage in goal-directed behaviour, but also consider *what* motivates individuals and *why* (McClelland, 1987; Deci & Ryan, 2000). Exploring differing motivational processes that enable the fulfilment of psychological needs, which in turn promotes the adoption of goals to meet these needs, enables research to better understand human growth, integrity and well-being (Dweck, 2017; Ryan & Deci, 2017). This approach led to the emergence of several multidimensional motivational theories, most notably, needs theory (McClelland, 1987), achievement goal theory (AGT; Nicholls, 1984), implicit theories (Dweck, 2016) and self-determination theory (SDT; Ryan & Deci, 2017). Although theoretically distinct, they nonetheless share similarities as to what drives engagement in goal directed behaviour, and why individuals are motivated by these psychological processes.

The first theory to conceptualise different motive systems and their impact on the energization of behaviour was needs theory (McClelland, 1987). McClelland's approach draws heavily from Freud's view of unconscious motives, and therefore the motivational processes are conceptualized and measured unconsciously. Furthermore, needs theory also acknowledged emerging theories of motivation, most notably Maslow (1954) and Rodgers (1951/2003), with an emphasis on the importance of increased creativity and growth (McClelland, 1987). Within needs theory, the three fundamental motive systems that energise, direct and select behaviour are the needs for power (nPower), achievement (nAch) and affiliation (nAff). nPower is the desire to control and exert influence over others, and is typified by displays of dominance or aggression,

and behaviour such as trying excessively to win arguments or collecting status symbols (McClelland, 1975). nAch is the innate disposition to seek and achieve challenging goals and take pride in one's accomplishments (Atkinson, 1957), while avoiding goals that are excessively difficult or too easily achieved (McClelland, Atkinson, Clark & Lowell, 1953). Finally, nAff is the desire to establish and maintain close bonds and a sense of belonging through individual relationships and social connectedness (Hofer & Hagemeyer, 2018; Koestner & McClelland, 1992). These needs are considered fundamental to all human motivation and are assumed to differ in strength between cultures and individuals. These individual differences are considered key in understanding human motives and behaviour (McClelland, Koestner & Weinberger, 1989).

Another theory adopting the concept of human needs to explain motivational processes is SDT. As an organismic theory, SDT is concerned with the processes that facilitate the capacity for psychological growth, integrity and well-being (Ryan & Deci, 2017). Humans are assumed to flourish to the extent that three basic psychological needs are satisfied; the needs for competence, autonomy and relatedness (Ryan, 1995). The satisfaction of basic psychological needs facilitates motivational processes that underpin engagement in goal-directed behaviour, explicated by a sub-theoretical framework of SDT, organismic integration theory (OIT, Ryan & Deci, 2017). OIT posits that, as growth-oriented individuals, humans will naturally internalise socially sanctioned behaviour. The key tenets of OIT, autonomous and controlled motivation, exist on a continuum, with the degree to which motivation is internalised and integrated with the sense of self representing the shift towards behaviour that is autonomously regulated (Ryan & Connell, 1989). Conversely when the internalisation process is forestalled or undermined, this represents controlled motivation (Ryan & Deci, 2000a). There is considerable variation within the processes that define autonomous and controlled motivation, and therefore distinct motivational processes are nested within this continuum. Intrinsic motivation represents behaviour that is fully autonomous and pursued for reasons of enjoyment or interest and is engaged in without the needs for separable consequences (Deci, 1975). The remaining motivational processes are classified as extrinsic motivation, whereby motivation is underpinned by separable outcomes (Ryan & Connell, 1989). Behaviour that is partially autonomous can either be integrated or identified regulation. Integrated regulation reflects the extent that important behaviours are integrated with aspects of the self and are reflective of an individual's values and identity (Deci,

Eghrari, Patrick, & Leone, 1994). Identified regulation is behaviour recognised as important and valuable and is therefore engaged in, and maintained with, greater volition (Ryan & Deci, 2017). Controlled motivation represents the least internalised motivational processes; introjected regulation characterises behaviour driven by internal feeling of guilt or pressure, is contingently administered by the individual onto themselves and therefore, although controlling, has nonetheless been partially internalised (Deci & Ryan, 2000). External regulation represents the most controlled form of motivation and is driven by specific external contingencies, such as the receipt of rewards or avoidance of punishments (Ryan & Deci, 2000a).

Two further multidimensional motivational theories that are conceptually linked and relate to motivation in achievement-oriented domains are AGT (Nicholls, 1989), and implicit theories (Dweck, 2016). These theories were developed concurrently as part of a collaborative research effort to better understand achievement-based motivation, and therefore share a close conceptual framework (Roberts, 2018). AGT is concerned with conceptions of ability, with the goal of demonstrating high rather than low ability (Nicholls, 1984). Achievement is demonstrated in one of two ways; by framing ability as either relative to one's own past performance or knowledge, or relative to the ability of others. Framing ability relative to the self is referred to as a task-oriented goal. In these instances, personal development and high effort are encouraged, mistakes are viewed as part of the learning process and cooperation with others is seen as facilitative. On the other hand, ego-orientation compares ability to a normative standard, mistakes are punished and competition between individuals is encouraged (Nicholls, 1989). Although the original focus of AGT was two constructs, in recent years this has increased to three (Elliot & Harackiewicz, 1996), four (Elliot, 1999) and the current theoretical expansion of six constructs incorporating valence, and delineating task- and ego-oriented goals into task, self and other orientations (Elliot, Murayama, & Pekrun, 2011).

Although sharing conceptual similarities with AGT, implicit theories (Dweck, 2016) differentiate from AGT by focussing on beliefs about the malleability of intelligence. Individuals who hold a fixed mindset view intelligence as static and are inclined to seek positive judgement from others. They are also less likely to engage in, and will have lower persistence during, a challenging task if their confidence in success is low (Elliott & Dweck, 1988). Conversely, individuals who hold a growth mindset consider intelligence to be malleable and are motivated to

increase competence. They are also more likely to seek challenges and have higher persistence in the face of adversity (Dweck & Leggett, 1988).

There exist conceptual similarities between needs theory, SDT, AGT and implicit theories; indeed, recent work has argued for closer empirical integration between these theories (Vansteenkiste, Lens, Elliot, Soenens & Mouratidis, 2014), and ambitious attempts have been made to fully integrate theories of motivation and personality (Dweck, 2017). However, despite their similarities, each theory offers a distinct perspective on motivational processes. Firstly, although nAch and nAff (and to a lesser extent nPower) share similarities with competence, relatedness and autonomy respectively (Schüler, Baumann, Chasiotis, Bender & Baum, 2018), they are theoretically distinct in how they explain human motivation. For SDT, it is not the basic psychological needs of competence, autonomy and relatedness per se that motivate behaviour. Rather, they are the essential nutriments that, when satisfied, facilitate the internalisation of behaviour; in other words, behaviour is pursued with greater autonomy (Ryan & Deci, 2000b). On the other hand, when there is lesser satisfaction of basic psychological needs, or when satisfaction is thwarted, this can foster more controlled functioning, undermining the internalisation of motivation (Ryan & Deci, 2017).

SDT also posits that the satisfaction of basic psychological needs influences growth, integrity and wellbeing. This process is irrespective of individual or cultural differences, which contrasts needs theory's assumed differences in the strength of needs between individuals and cultures (Deci & Ryan, 2000; Schüler et al., 2018). Furthermore, all of these motivational theories assume that individuals are not necessarily conscious of their motivational processes and how these processes affect behaviour (Deci & Ryan, 2000; Ryan & Deci, 2017). However, SDT, AGT and implicit theories assess motivational processes explicitly, typically using self-report measures. This contrasts with needs theory, where motivational processes are conceived and assessed as implicit and driven unconsciously (McClelland, 1987).

There is also a differentiation between higher and lower quality motivational processes. Implicit theories, AGT and SDT have a greater focus on the importance of the quality of motivation, versus the quantity. For example, higher quality motivation (i.e., autonomous motivation, task-oriented, growth mindset) are not only associated with improved outcomes but are also viewed as adaptive and growth-oriented (Ryan & Deci, 2017; Roberts, 2018). In contrast, although lower quality motivation (i.e., controlled motivation, ego-oriented, fixed

mindset) can lead to positive behavioural outcomes when there is a sufficient quantity of motivation, it is nonetheless considered maladaptive (Deci, Koestner & Ryan, 1999). For example, the pursuit of external rewards, such as financial rewards or prestige, may well yield positive short-term benefits by motivating an individual to achieve their goals (Gagné, Deci, & Ryan, 2017). However, it is associated with psychological dysregulation, poorer mental health and undermines an individual's personal development and growth (Ng et al., 2012). In contrast, although needs theory differentiates between motivational processes, the qualitative influence on adaptive human functioning is not emphasised, although it may be implied. For example, during an academic year, students who were high in nAff and low in nPower experienced an adaptive immune response compared with students who were low in nAff and high in nPower, with the effect more pronounced during an examination period (Jemmott et al., 1983). Thus, the higher quality adaptive process evident in this situation are dependent on the contrasting dominance of the two needs and is also situation-dependent.

To illustrate the qualitatively distinct reasons of what motivates behaviour and why, consider four individuals, who each take a 30 minute walk a day. Stephen is a 48-year-old male who thoroughly enjoys walking and takes a 30-minute walk with his partner every morning before starting work. He does this purely for personal pleasure, as he enjoys being out in the fresh air and observing nature. Catherine is a 15-year old girl who walks her dog every day; however, she does not really enjoy walking and only does so as she feels guilty if she does not exercise her dog. Jessica is a 25-year-old female who uses her smartphone and a wearable fitness device to track her activity. She recognises the importance of being fit and healthy, and therefore sets a goal of five thousand steps per day. However, she often feels frustrated when she compares her performance to her friends on social media, and only feels truly satisfied when she outperforms them. Lastly, Mohammed is a 55-year-old male who is overweight and requires an operation. Mohammed dislikes walking, and he only goes on his afternoon walk as a weight loss intervention, as his doctor believes this will give him the best chance of a successful operation and long-term recovery. However, he believes exercising will not help – he believes his weight is determined by genetic factors and changing his behaviour will not help him lose weight.

These individuals engage in similar levels of physical activity; however, despite achieving similar behavioural outcomes, their reasons for doing so are distinctly different. Stephen's motivation is the highest quality as the reasons underpinning his behaviour are

intrinsically motivated, that is, the activity is performed purely for the enjoyment and satisfaction, with an absence of rewards or re-enforcement (White, 1959). Moreover, he enjoyed spending quality time with his partner, and walking also therefore satisfies his nAff (McClelland, 1987). On the other hand, Mohammed is motivated by the prospect of receiving a vital operation, as well as external pressure by his doctor, and is therefore extrinsically motivated (De Charms, 1968; Skinner, 1953). Extrinsic motivation encompasses activities that are undertaken purely to attain a separable reward or outcome, and therefore Mohammed's motivation is externally regulated. Furthermore, he believes his weight is determined by his genetics, and behavioural improvements will not help him to lose weight, thus displaying a fixed mindset (Dweck, 2016). Catherine's motivation is also controlled; it is introjected as it is internally regulated by feelings of guilt about not walking her dog (Ryan & Deci, 2017). Finally, Jessica's identified motivation stems from her belief in the importance of engaging in healthy behaviour (Deci et al., 1994). However, her motivation is also ego-oriented (Nicholls, 1984); she only feels satisfied when she achieves more steps per-day than her friends. These examples demonstrate qualitatively distinct reasons that explain what motivates individuals, and why. In addition, they emphasise that individuals can hold different types of motivation for performing the same task. The source of an individual's motivation is important as research has demonstrated that the reasons motivating behaviour can have important implications for the psychological health and wellbeing of the individual (Deci & Ryan, 2008).

Motivation of a higher quality is associated with a wide-range of health-related and adaptive psychological outcomes. For example, a recent meta-analysis found that intrinsic motivation was associated with improved physical and mental health, and conversely, extrinsic motivation was associated with poorer mental health and had no effect on improved physical health (Ng et al., 2012). This therefore suggests that higher quality motivation is a key determinate not only in predicting improved physical health, but also improved mental health. On the other hand, motivation that is conceptualised as lower quality has a deleterious effect on mental health, and no effect on positive physical health (Ng et al., 2012). Furthermore, although qualitative and quantitative motivation predict similar levels of performance, there are subtle differences in the performance criterion. For example, motivation that is either intrinsic or extrinsic in origin predicts similar levels of performance. However, intrinsic motivation better predicts higher quality performance, such as engagement with complex tasks, whereas extrinsic

motivation is a better predictor of performance on less complex tasks with a focus on outcomes (Cerasoli, Nicklin & Ford, 2014). These distinctions are important as they allow researchers differentiate between types of motivation that promote personal growth and greater wellbeing from those that may have positive behavioural outcomes but may also have deleterious psychological and physiological consequences. The greater level of depth and global orientation provided by SDT compared with domain-oriented AGT and implicit theories, and the lesser emphasis on the quality of motivation as an important determinant of improved well-being evident in needs theory, led to the present thesis focusing on SDT as a guiding framework.

There is strong evidence that higher quality self-determined motivation to engage in physical activity leads to improved psychological wellbeing (Ng et al., 2012). However, what is less clear is if there is a direct effect of motivation on physiological health beyond that which is explained by engagement with the activity itself. For example, when two individuals engage in identical levels of physical activity, do the individuals motivated by higher quality reasons benefit from greater health? Or conversely, do those motivated by lower quality reasons pay greater psychophysiological health costs? Although progress has been made to address these questions using self-report and behavioural measures of health and well-being, far less emphasis has been placed on the examination of biological and physiological markers. A recent review examining the associations between motivational neurological processes elucidated the importance of extending research in this area (Di Domenico & Ryan, 2017). First, biological processes mediate the relationship between motivational processes and behaviour. For example, if we are late for the train and wish to arrive home on time, we are dependent on our physiological processes to meet this goal. Second, examination of biological processes allows the observation of internal processes that are not accessible via self-report measures or behavioural observations. For example, an individual can potentially provide socially desirable responses if they are assessed using self-report measures; however, biological measures are not so easily manipulated. Third, measurement of biological functioning allows for a more fine-grained measurement than behavioural or self-report measures. For example, cardiovascular reactivity has been indexed as a measure of effort intensity (Wright & Gendolla, 2012). Using this approach, the effort expended on a cognitive task can be accurately measured to control for individual differences in task motivation. Exploration of physiological markers therefore provides a critical empirical link between motivation and behavioural processes. Moreover, this

research is important for affirming the validity of motivational research that has predominately relied on observational and self-report measures of wellbeing and health. Thus, the primary aim of this thesis was to explore the relationship between the quality of motivation and biological markers implicated with adaptive human functioning and health related-outcomes. This exploration began by systematically reviewing the extant literature between multidimensional motivational processes and activation of the endocrine system

The Endocrine System

Endocrinology represents a longstanding important area of psychological research (e.g., Selye, 1950). The endocrine system is made up of glands including adrenal, parathyroid, pineal, pituitary and thyroid glands. As one of the body's main systems, the endocrine system contributes to maintaining homeostasis by regulating the activity of the cells throughout the body. This is accomplished by the endocrine glands secreting hormones into interstitial fluids. These hormones then diffuse into the blood stream and are carried to target cells throughout the body, binding to the receptors of the target cells. Hormones can only bind to the receptors that are specific to that hormone. Furthermore, the receptors are sensitive to greater hormonal activity and can be downregulated if there is an excess of hormones, or upregulated if there is a deficiency. The endocrine system contributes to homeostasis by regulating activity of several bodily functions including metabolism, driving developmental and reproductive processes; influencing circadian rhythms; and the resistance to stress through the release of cortisol (Tortora & Derrickson, 2017). It is this later proposition that this thesis explored in relation to motivational processes.

As a key hormone associated with psychological, physiological and physical health functioning, cortisol has been investigated in hundreds of studies over the past half-century (Dickerson & Kemeny, 2004). In particular, there has been a focus on cortisol as a marker of stress reactivity, with key reviews and meta-analysis investigating acute (Dickerson & Kemeny, 2004) and chronic stress responses (Miller, Chen & Zhou, 2007). It has been found that cortisol can be triggered by physical factors such as surgery, toxins, wounding, infections and fever (Tortora & Derrickson, 2017). Furthermore, cortisol can be triggered by psychological experiences such as social evaluation (Dickerson & Kemeny, 2004), post-traumatic stress disorder (Pitman et al., 2012) or unemployment (Arnetz et al., 1991). The psychological element leading to a cortisol response can be perceived differently by different people, and even by the

same person at different times. For example, while most people might be excited by the challenge of starting a new job, others may be overwhelmed by negative thoughts relating to their ability to cope.

If our evaluation of a situation is that it is within our coping resources, homeostasis is maintained and body functions normally. However, if the perception of demands exceeds coping resources, then a sequence of physiological changes occurs, known as a general adaptive syndrome (Selye, 1936). In this adaptive response, the hypothalamus and autonomic nervous system immediately mobilise resources to enable a physical response. This involves large amounts of oxygen and glucose being made available for the vital organs, including brain, heart and skeletal muscles. As part of the secondary response, also known as the resistance reaction, the hypothalamus pituitary adrenal (HPA) axis mobilises growth hormone–releasing hormones (GHRH), corticotropin-releasing hormones (CRH), and thyrotropin-releasing hormones (TRH). The hormone CRH subsequently binds to CRH receptors of the cells, and adrenocorticotropic hormone (ACTH) is released, which in turn stimulates the release of cortisol via the adrenal cortex. Cortisol subsequently carries out several functions, including gluconeogenesis, lipolysis and catabolism of proteins into amino acids. The body subsequently uses these resources to repair damaged tissue, or to produce more adenosine triphosphate (ATP), the body's key currency used in energy transfer. Once sufficient resources have been accumulated to meet the demand, cortisol supresses CRH and the stress response is eventually alleviated. Cortisol therefore plays a key role in counteracting situational demands and is frequently used in psychophysiological research as a marker of HPA activity.

Despite their protective effects, glucocorticoids, including cortisol, are also responsible for suppression of the immune system. Glucocorticoids exert anti-inflammatory effects, attenuate tissue repair and wound healing, and depress immune functioning (Segerstrom & Miller, 2004). Moreover, prolonged periods of cortisol reactivity can lead to several stress-related disorders including asthma, rheumatoid arthritis, ulcerative colitis, hypertension, migraine headaches, anxiety and depression (Tortora & Derrickson, 2017). Harmful and long-lasting effects associated with a frequent cortisol reactivity represent an important area of psychological research. However, exploration of cortisol-related physiological mechanisms and motivation is still in its infancy.

The idea that motivational processes are linked to physiological responses is not a new one (e.g., Cannon, 1927). Indeed, there is a large volume of work implicating increased motivation with cardiovascular responses (Wright & Gendolla, 2012). However, in these specific contexts, motivation is explored as a unidimensional construct whereby a greater investment of resources (i.e., effort) leads to improved behavioural outcomes. Furthermore, increased effort is also associated with an increased physiological response which is reflective of the task demands (Richter, Gendolla & Wright, 2016). For example, motivational intensity theory supports the idea that individuals will expend effort only to the degree that is needed, and when the expenditure yields a benefit (Brehm & Self, 1989). Thus, effort (i.e., quantity of motivation) increases in a linear fashion in response to a task difficulty provided the benefits are deemed attractive enough; typically manipulated using monetary incentives (e.g., Richter & Gendolla, 2009). To validate this proposition, researchers have indexed cardiovascular reactivity as a marker of effort, with pre-ejection period and systolic blood pressure increasing in response to task difficulty (Richter, Friedrich & Gendolla, 2008). Thus, individuals are externally motivated by increased task demands provided rewards are deemed sufficient.

The effects of the quality of motivation on the psychological processes are less well understood. However, recent studies have been initiated to explore the endocrine system as a potential marker of multidimensional motivational theories. Although research in this area is sparse, most of the work has employed experimental designs, comparing the effects of higher versus lower quality motivation. For example, Reeve & Tseng (2011) compared the effect of manipulating the motivational environment during a problem-solving task. Compared with a neutral condition, participants who were exposed to a controlling environment experienced a significant increase in cortisol, while those in the autonomy-supportive condition experienced a decrease. Two further studies adopted a similar approach, measuring cortisol during a juggling task. Participants were exposed to the AGT constructs of either a task/caring or an ego-oriented environment in a population of university undergraduates (Hogue, Fry, Fry & Pressman, 2013) and adolescent schoolchildren (Hogue, Fry & Fry, 2017). In both studies, there was a significant difference between conditions over time, with participants in the ego-oriented environment displaying an increase in cortisol, with cortisol decreasing in the task/caring environment. Further elucidating this proposition, schoolchildren that were exposed to an incremental theory intervention experienced a significantly decreased cortisol response when compared with a

control group. This finding was offered partial support in a pre-registered double-blind experimental intervention (Yeager, Lee & Jamieson, 2017).

Research in this area extends to several markers of endocrine functioning. Furthermore, the research extends to markers that are not part of the endocrine system but are directly regulated by it. Several experimental studies investigating needs theory have demonstrated a link between winning a contest and increased testosterone for participants high in nPower (Stanton & Schultheiss, 2009). Furthermore, participants high in nAff, but low in nPower, experienced improved immune functioning during times of stress such as examination periods (Jemmott et al., 1983; McClelland, Ross, & Patel, 1985), with experimental research supporting this association (McClelland & Kirshnit, 1988). The adaptive effects of the motivational processes have also been observed in studies grounded in other theoretical positions including SDT (Bartholomew et al., 2011), thus validating the idea that higher quality motivation is associated with improved wellbeing.

A key limitation of the extant literature is the fragmentation of research across theoretical boundaries. This presents a problem in ascertaining the current state of the science and identifying areas ripe for future exploration. A trans-theoretical review will provide a complete overview of the relationships between motivational theories and endocrine-related responses. This will help to advance motivation and psychophysiological science by clarifying the relationships between motivational constructs and physiological responses within theoretical boundaries. Furthermore, it will enable comparison of conceptual similarities between theories when exploring similar research questions and physiological outcomes. For example, as highlighted in this introduction, SDT, AGT, implicit theories and needs theory share theoretical overlap. Comparison between theories will provide a comprehensive overview of the theoretical and conceptual similarities, methods employed and results. Moreover, the examination of the relationship between motivation and physiological responses has come under scrutiny due to weak scientific practices leading to unwarranted conclusions (Richter & Slade, 2017). A systematic review can illuminate instances where such practices have occurred. Chapter Two will address these issues by presenting a systematic review of multidimensional motivation theories and psychophysiological responses associated with the activation of the endocrine system.

Self-Regulation, Motivational Processes and Cortisol Reactivity

A key limitation identified in the systematic review presented in Chapter Two is the absence of performance measures when examining the motivational effects on cortisol. This is important – a key tenet of SDT is that it is associated with improved well-being and optimal behavioural outcomes. However, despite this assertion, there have been few attempts to test these assertions concurrently using physiological outcomes to assess the potential implications for health and well-being. Although there is a variety of ways to investigate motivational processes and performance-related outcomes, in the context of the present body of work, integration of the performance measures that require self-regulation offer distinct advantages, and these will be elucidated in the following section. Self-regulation is a broad goal-directed motivational process that monitors progress and adjusts regulate behaviour in the pursuit of incentives or the achievement of goals (Carver & Scheier, 2018; Baumeister & Vohs, 2016a). Self-regulation and motivational processes are associated with stress and coping, with cortisol acknowledged as a key psychophysiological pathway of influence (Carver & Vargas, 2011). It is accepted by prominent motivational theorists that human behaviour is energised and directed by the pursuit of goals (Bandura, 1986; Dweck, 2017; McClelland, 1987; Ryan & Deci, 2017). The motivational processes that energise movement towards goals are bounded by an individual's access to, and availability of, resources (Hobfoll, 1989). In this context, self-regulation describes the process of monitoring the individual's progress towards goals, the attainability of the goal, and the availability of resources (Brehm & Self, 1989; Holahan, Moos, Holahan & Cronkite, 1999). Therefore, according to Carver and Vargas, it is the motivational processes that drive goal-directed behaviours, and self-regulation that organises and monitors progression.

When an individual perceives a threat to goal achievement, this activates the potential for a cortisol response. The relationship between the situational demands and a cortisol response is moderated by the availability of coping resources (Carver & Vargas, 2011). Thus, a cortisol response only occurs when an individual's appraisal of situational demands exceeds coping resources, or sufficient coping resources are unavailable. This framework provides a theoretical overview of the relationship between motivation, self-regulation and cortisol. However, despite the importance of motivational processes in Carver and Vargas' theoretical model, few attempts have been made to integrate multidimensional motivational theories within this framework (Elliot, Thrash & Murayama, 2011).

A self-regulatory process that has integrated multidimensional motivational theories into its theoretical framework is self-control. Self-regulation and self-control are often referred to interchangeably in the literature. They share a close conceptual and theoretical framework and have ostensibly been used to describe the same theory (Baumeister & Vohs, 2016a; Baumeister, Vohs & Tice, 2007). Despite the similarity with self-regulation, self-control is more frequently used to describe the mental process of overriding impulses or altering thoughts, emotions or behaviour (Inzlicht & Schmeichel, 2012), thus bringing them in line with ideals, morals, values and social expectations (Baumiester et al., 2007), and can refer to trait processes (Baumeister & Vohs, 2016a). Research into self-control was stimulated by a series of experiments demonstrating that children held individual differences in their ability to exert self-control by resisting eating marshmallows (Mischel, 1961). Subsequent research demonstrated that four-year-old children with higher levels of self-control were more socially competent, had higher academic achievement, and coped better with stress during adolescence (Mischel, Shoda & Rodriguez, 1989; Shoda, Mischel & Peake, 1990). Moreover, these findings were supported in a 40-year follow-up of participants who took part in the original research (Casey et al., 2011). Self-control has also received support as a stronger predictor of academic achievement than IQ (Duckworth & Seligman, 2005), further highlighting the potential importance of self-control in goal-directed behaviour.

Contemporary self-control research was stimulated by an elegant theory proposing self-control as a limited resource (Baumeister, Heatherton & Tice, 1994, Baumeister, Bratslavsky, Muraven & Tice, 1998). The strength model of self-control suggests an individual's capacity to exert self-control is contingent on a finite resource. Tasks requiring self-control deplete the limited resource, leading to the deterioration of performance on further tasks drawing on self-control resources. The depletion of self-control, also known as ego depletion, is typically measured using the sequential-task paradigm (Baumeister et al., 1998). This experimental design requires and experimental group to perform two different tasks requiring the exertion of self-control. A control group performs an identical second task to the experimental group; however, the first task, while conceptually similar to the one performed by the experimental group, does not require self-control. Performance results between the groups on the second task is then compared, with the difference in performance assumed to represent the exertion of self-control. The strength model of self-control has stimulated a body of research that examines a wide range

of tasks related to the exertion of self-control, with a meta-analysis including over 100 studies finding a moderate effect size for ego depletion effect (d = .62; Hagger, Wood, Stiff & Chatzisarantis, 2010). However, the size of the effect reported by Hagger and colleagues is disputed. Further investigation has questioned the reported effect due to the absence of null findings attributable to publication bias (Carter & McCullough, 2013). A subsequent meta-analysis conducted a series of statistical analyses controlling for publication bias and found little evidence for the existence of ego depletion (Carter, Kofler, Forster & McCullough, 2015). Furthermore, a high-powered multi-lab preregistered replication also failed to find evidence of the ego depletion effect (Hagger et al., 2016), casting further doubt over the existence of the effect.

Although the debate around the strength model of self-control and the ego depletion effect continues (Baumeister & Vohs, 2016a; Blázquez, Botella & Suero, 2017), the controversy has stimulated scientists to propose modified self-control theories. Alternative models suggest that self-control failure may not be a question of limited resources, but how they are allocated (Beedie & Lane, 2012), or that computational mechanisms are deployed as a function of the opportunity costs of task performance (Kurzban, Duckworth, Kable & Myers, 2013). A further model that has particular contextual relevance to the present research is the shifting priorities model of self-control (Inzlicht & Schmeichel, 2016; Milyavskaya & Inzlicht, 2016). This view proposes exertion of self-control during an initial task compromises success on further tasks due to reduced attention and motivation to exert self-control. Moreover, the salience of motivation and attention towards external rewards and impulses is assumed to increase during this process.

Research linking motivational processes and self-control was stimulated by a series of experiments that manipulated motivation towards tasks requiring self-control. Initial research found participants performed better on self-control tasks when they were provided with a persuasive rationale; when they believed the task could benefit them; or when offered monetary rewards (Muraven & Slessareva, 2003). Further research has explored self-control from a variety of theoretical multidimensional motivational perspectives. Studies grounded in implicit theories (Dweck, 2016) demonstrated that belief in self-control (viz. willpower) as non-limited resource leads to improved self-control performance compared with individuals who held beliefs as limited (Job, Bernecker, Miketta & Friese, 2015; Job, Dweck & Dalton, 2010; Job, Walton, Bernecker & Dweck, 2014). In addition, higher implicit needs for power and achievement

(McClelland, 1987) have also shown to improve self-control performance (Gröpel & Kehr, 2014).

The effects of autonomous and controlled motivation on self-control have also been investigated. In the first study to test this proposition, autonomous motivation mediated the ego depletion effect, with participants displaying increased persistence, vitality and energy, when compared to a controlled motivation condition (Moller, Deci & Ryan, 2006). This effect was supported by research demonstrating performance contingent rewards led to greater ego depletion than non-contingent rewards, with autonomy support mediating the relationship (Muraven, Rosman & Gagne, 2007). This finding was replicated and extended by demonstrating improved self-control performance when task instruction was delivered using autonomy-supportive language, compared with being instructed with more controlling language (Muraven, Gagne & Rosman, 2008). Finally, participants who held more autonomous reasons for resisting eating cookies performed better on a subsequent self-control task (Muraven, 2008). Thus, the published literature strongly supports the idea of autonomous motivation mediating the ego depletion effect.

Further research in this area has refined the idea of motivational processes and the effect on self-control. Vohs, Baumeister and Schmeichel (2012) found that autonomy support and beliefs in unlimited willpower improved self-control performance within the sequential-task paradigm. However, they also found this effect disappeared when exposed to multiple self-control tasks. This finding was replicated and extended, with autonomy support enhancing short- but not long-term self-control performance, with participants conserving self-control resources when anticipating future self-control tasks (Graham, Bray & Martin-Ginis, 2014). Therefore, it seems that autonomous motivation is an important mechanism in mediating self-control performance, however the effects themselves are time-limited. Furthermore, self-regulation would appear to be an important process in monitoring and adjusting behaviour according to the demands of the present tasks, and expectation of future task demands (e.g., Graham et al., 2014; Vohs et al., 2012).

Recent theoretical and empirical work has begun to shift away from explicit tests of the ego depletion effect towards exploration of the mechanisms that may be responsible for many of the contrasting findings in this area (e.g., Kotabe & Hofmann, 2015; Milyavskaya, Inzlicht, Hope & Koestner, 2015). However, a relatively unexplored area of self-control and ego depletion is the

concurrent response of cortisol. There is a coherent theoretical argument for an association between cortisol, motivation and self-regulation (Carver & Vargas, 2011). Furthermore, empirical evidence supports the link between motivation and cortisol, and motivation and self-control. Despite the scientific rationale for exploring the relationship between self-control and cortisol, there is relatively little investigation in this area. The only study that explicitly tested the association between self-control and cortisol found trait self-control predicted an improved circadian cortisol profile via stabilising emotional states (Daly, Baumeister, Delaney & MacLachlan, 2012). Although this appears to be the only study exploring self-control and cortisol, there are studies offering proxy support. For example, increased expenditure of psychosocial resources was associated with significantly lower cortisol reactivity following a stress task (Taylor et al., 2008). Furthermore, there are theoretical and empirical associations between self-control and self-report measures of stress. Stress is assumed to exert a negative effect on self-control (Baumeister et al., 1994; Muraven & Baumeister, 2000). This has been tested in relation to exam stress and performance on the sequential-task paradigm (Oaten & Cheng, 2005). Stress has also been demonstrated to reduce self-control in adolescents (Duckworth, Kim & Tsukayama, 2013) and adult populations (Hamilton, Sinha & Potenza, 2014). Finally, recent evidence suggests a bidirectional effect, as individuals who performed better during the Stroop task, a validated measure of self-control, tended to use self-control to manage stressful situations (Klein, Liu, Diehl & Robinson, 2017). Based on the theoretical and empirical associations between motivational processes, self-control and cortisol, Chapters Four will experimentally manipulate self-determined motivation, with the aim of investigating the concurrent effects on self-control performance and the cortisol response.

A limitation of the method used to assess self-control in Chapter Three was the assessment of ego depletion using the sequential-task paradigm. As previously discussed, despite meta-analytical support for the existence of the ego-depletion effect (Hagger et al., 2010), support is equivocal (Carter & McCullough, 2014; Carter et al., 2015). However, issues with replication and the existence and size of effects extend beyond self-control and ego-depletion. The replication crisis in psychology, while damaging for the credibility of the science, has led to a wide-ranging discussion about what constitutes good science, and recommended practices for mitigating issues of selective reporting (Simmons, Nelson & Simonsohn, 2011; Finkel, Eastwisk, Reis, 2015). Addressing these concerns, psychologists have argued strongly for replication to

confirm the accuracy of empirical findings (Klein et al., 2014). Replication to ensure the robustness of results is an important aspect of the scientific process of discovery. Results that are incorrectly thought to support a hypothesis (i.e., false positives) are difficult to weed out once published, can waste valuable resources as time is invested trying to replicate, and risk undermining the credibility of science (Simmons et al., 2011). Considering the issues surrounding the replication crisis, and the increased scrutiny on ego depletion, it is important to replicate findings that may have key implications for future ego depletion research. The aim of Chapter Four was therefore to replicate and extend the results of Chapter Three, and where possible address and refine the acknowledged limitations of the original study design.

Epigenetics

This thesis has explored associations between motivational processes and acute endocrine-related responses. However, it would flesh out the underlying science to examine associations between motivational processes and biological markers that are more stable over time and may have implications for optimal human functioning and chronic health conditions. For such an exploration, as highlighted in Chapter Two, the endocrine system is unreliable for cross-sectional exploratory work. The endocrine system is sensitive to moment-to-moment variation including diurnal variation, consumption of stimulants, and perceptions of stress (Dickerson & Kemeny, 2004; Smyth, Hucklebridge, Thorn, Evans, & Clow, 2013). The final experimental chapter of this thesis therefore focused on epigenetics - a novel and as-yet unexplored area of biology for motivational science. Epigenetic modifications are sensitive to psychological factors, are associated with long-term behavioural change, and remain stable over time (Cole et al., 2007; Jones et al., 2018). Furthermore, the investigation of the potential implications of healthy behaviour in affecting epigenetic processes is still very much in its infancy. Epigenetics therefore represents an ideal lens through which to explore motivational processes and healthy behaviour engaged in over the long-term, and the potential implications for adaptive human functioning.

Epigenetics has been described as one of the most exciting areas of contemporary biology and has captured the imagination of the press and popular media (Bird, 2007). The scientific term *epigenetics* was first coined in pioneering work exploring the association between the genotype and phenotype (Waddington, 1942). The genotype represents an organism's inherited genetic makeup; its sequence of nucleotide bases in its DNA. The phenotype represents the

organism's physical traits and arises from a wide-variety of biological mechanisms. Over the last three decades the term epigenetics has been used to broadly explain how the environment can affect gene expression, however, the precise meaning of epigenetics has undergone several revisions that mirror the evolution of the underlying science (e.g., Holliday, 1994). The generally accepted contemporary definition of epigenetics is "the study of changes in gene function that are mitotically and/or meiotically heritable and that do not entail a change in the sequence of DNA" (Wu & Morris, 2001, p. 1104). In other words, chemical modifications that do not alter the DNA sequence, but alter the expression of genes, can be passed through generations of cell division (i.e., mitosis) or through sexual reproduction (i.e., meiosis).

Although epigenetic mechanisms are studied at the genetic level, their influence is often evident as observable physical manifestations. As they are a result of a split of the same embryo in the womb, monozygotic (identical) twins retain identical DNA throughout their lives and inherit the same genetic modifications. However, exposure to different environments, lifestyles and experiences leads to epigenetic drift (Teschendorff, West & Beck, 2013). This effect is exacerbated when monozygotic twins have been raised separately, with observable differences most evident in old age as the impact of environmental differences accumulates over time (Fraga et al., 2005). In addition, to physical differences, there are also noticeable differences in susceptibility to psychiatric conditions including schizophrenia and depression (Cardno, Rijsdijk, Sham, Murray & McGuffin, 2002).

There are several epigenetic mechanisms implicated in the alteration of gene expression including, but not limited to, histone modification, methylation of messenger ribonucleic acid (mRNA) and the most frequently studied, and the focus of the present investigation, DNA methylation (Henikoff & Greally, 2016). DNA methylation is characterized by the addition of a methyl group to a cytosine nucleotide, creating 5-methylcytosine (5mC), most commonly at cytosine-phosphate-guanine (CpG) sites. The effect of DNA methylation commonly results in the transcriptional silencing of gene expression around the transcription start site and the first exon, leading to the gene being "switched off" and consequently expression is altered (Brenet et al., 2011). Genetic modifications that influence gene expression without altering the DNA sequence represent the essence of epigenetics.

Epigenetics has opened a promising line of investigation for health scientists, because alterations to gene expression have been associated with the pathogenesis of, and susceptibility

to, disease (Cookson, Liang, Abecasis, Moffatt & Lathrop, 2009; Emilsson et al., 2008). As a result, the identification of modifiable factors that can positively influence disease-related epigenetic processes represents a worthwhile scientific endeavour. Behavioural risk factors such as diet, physical activity and lifestyle choices have all been linked with epigenetic modifications (Bjornsson, Fallin & Feinberg, 2004; Jaenisch & Bird, 2003). However, psychological experiences, as possible sources of epigenetic changes, have been investigated to a much lesser degree, despite significant potential to enhance understanding of how psychological experiences shape human development.

Although empirical research examining psychological variables and DNA methylation is very much in its infancy, research has revealed encouraging findings. For example, social connectedness is assumed to purvey a systematic difference in the expression of bacterial and viral immune responses (Cole, 2013). Exploring this proposition, research has supported the subjective experience of social isolation, and not objective social isolation, is associated with the up-regulation of pro-inflammatory genes, and downregulation of anti-viral and antibody regulated genes (Cole et al., 2007). Further research of psychological processes imply that stress, coping and self-regulation are all potential epigenetic mechanisms. Associations have been found between life stress, post-traumatic stress disorder, child abuse and global DNA methylation in African American subjects (Smith et al., 2011). Perceived stress, cortisol output, and socioeconomic status (SES) have also been implicated as psychological mechanisms associated with DNA methylation (Lam et al., 2013). Self-regulation has also been implicated as a mechanism for global methylation. Miller, Yu, Chen and Brody (2015) found that among high-SES individuals, better self-regulation predicted more favourable psychological and epigenetic aging. Among individuals of low-SES, higher self-regulation also predicted more favourable psychological outcomes, however, it came at a cost of faster epigenetic aging. This growing body of evidence supports the idea that the subjective experience of psychological factors such as perceived stress or the social environment may play a prominent role in adaptive genetic modifications and may be more important than behavioural measures.

Epigenetic processes are assumed to exert wear and tear on the human body through disruption of immunological and inflammatory processes (Jones, Moore & Kobor, 2018). For example, in a study examining the impact of SES on transcriptional profiling, those individuals raised in low-SES environments were more susceptible to down-regulation of glucocorticoid

receptors, resulting in increased daily cortisol output and increased expression of proinflammatory cytokines (Miller, Chen & Cole, 2009). Although negative environmental experiences are associated with disrupted immune functioning via epigenetic processes, these processes may be modifiable by improved healthy behaviour, specifically, physical activity. A recent literature review examined physical activity and the methylation of inflammation-related epigenetic markers (Horsburgh, Robson-Ansley, Adams & Smith, 2015). Horsburgh and colleagues reviewed both acute and habitual physical activity, and the impact of exercise interventions on DNA methylation and disease. Summarising the review findings, inflammatory responses to acute exercise were a likely mechanism in the regulation of DNA methylation. Moreover, physical activity interventions of between six to 12 months were sufficient to methylate genes associated with aging, type two diabetes and cancer. Due to the limited number of published studies, no firm conclusions could be drawn between habitual physical activity and DNA methylation of inflammation-related epigenetic markers. Only one study in the literature review explored associations between methylation of the *TNF* promoter and physical activity (Shaw, Leung, Tapp, Fitzpatrick, Saxton & Belshaw, 2014). The cohort study followed an elderly population at baseline and 10-year follow-up, with physical activity positively associated with increased methylation of the *TNF* promoter, an area of the genome responsible for the transcription of TNFα. It is this loci-specific transcription site that will form the basis of the present investigation.

The proinflammatory cytokine TNFα is responsible for several functions including regulating the response to infection (Vassalli, 1992). It also helps protect against fever and viruses, as well as regulating tissue glucose and the metabolism of fat. However, elevated levels of TNFα are associated with several noncommunicable diseases such as rheumatoid arthritis, Crohn's Disease, type one diabetes, as well as noncommunicable risk factors such as obesity (Arroyo-Jousse Garcia-Diaz, Codner Pérez-Bravo, 2016; Maxwell et al., 2008; Van Deventer, 1997; Ye, 2008). TNFα is primarily regulated at the transcriptional and post-transcriptional level (Sariban, Imamura, Luebbers & Kufe, 1988), and therefore examining potential mechanisms that can help regulate circulatory levels of TNFα, via DNA methylation, represents a promising line of enquiry to help manage health-related disorders.

Although circulatory levels of TNFα can be managed using medication, research has also demonstrated that TNFα can be modified in response to physical activity. In the first study to examine this relationship, a 150-minute running test increased plasma TNFα, with levels returning to pre-test levels within 48 hours (Dufaux & Order, 1989). Increased plasma TNFα has been reported on ergometer tasks (Goebel, Mills, Irwin & Ziegler, 2000); however, other studies have reported TNFα increases at 70% of Vo2max, but not 50%, suggesting that TNFα may only be affected by vigorous physical activity (Kimura, Suzui, Nagao, & Matsumoko, 2001). Despite support for short-term increases in TNFα in response to acute physical activity, results are not equivocal with some studies reporting no effect (see: Gökbel et al., 2012; Febbraio, Steensberg, Starkie, McConell & Kingwell, 2003; Haahr et al., 1991). In summary, there is tentative evidence to suggest that circulatory levels of TNFα may be increased by acute high-intensity physical activity, with levels returning to normal within a relatively short timeframe.

Although the short-term effects of physical activity on TNFα have revealed mixed findings, the long-term effects of physical activity on plasma levels of TNFα were more promising. A quasi-experimental study featuring pregnant women observed increased plasma levels of TNFα during pregnancy; however, maintaining exercise during the early stages of pregnancy attenuated this effect (Clapp & Kiess, 2000). In clinical populations, it is the contextual relevance of TNFα that is important, not absolute increases or decreases. For example, increased expression of TNFα via mRNA in pregnant women is associated with increased insulin resistance (Clapp & Kiess, 2000); a risk factor for heart disease and diabetes. This association was also demonstrated in an obese population where a long-term physical activity intervention decreased TNFα system activity, which coincided with increased insulin sensitivity; a mechanism that reduces the risk of diabetes (Straiczkowski et al., 2001). Similar results have been found in populations where increases in TNFα have been proposed as a causal factor for disease. Sarcopenia is an age-related muscle wasting disease causing considerable morbidity with increased levels of TNFα linked to suppression of protein synthesis and skeletal muscle breakdown (Goodman, 1991; Sakurai, Zhang, & Wolfe, 1996). In a three-month study where the effect of TNFa on sarcopenia was examined in an elderly population, a resistance training programme reduced both circulatory TNFα levels and muscle wastage, suggesting TNFα may attenuate age-related muscle wasting (Greiwe, Cheng, Rubin, Yarasheski &

Semenkovich, 2001). To summarise, extant research suggests long-term physical activity programmes of moderate intensity can lead to reduced plasma levels of TNFα within populations suffering from diseases that are susceptible to, and a consequence of, increased levels of TNFα. There is a relatively weaker body of evidence that suggests acute physical activity provokes a short-term increase in circulatory TNFα. Furthermore, increases are potentially related to acute exercise intensity, with TNFα levels returning to pre-exercise levels within a relatively short timeframe.

It has been suggested that the increased inflammatory response to acute physical activity may act as a mechanism in downregulating long-term inflammatory expression (Gleeson et al., 2011). This provokes the suggestion that more fundamental changes in physiology, and potentially epigenetics, may be implicated in the regulation of circulatory markers of immunity including TNFα. A limitation of contemporary research exploring the immune response is an over-reliance on research examining circulatory plasma levels (Gleeson et al., 2011), highlighting a need to examine causal factors that regulate the immune response. Regulation of circulatory TNFα is primarily at the post-transcriptional and transcriptional level (Sariban et al., 1988). Therefore, examining epigenetic processes of the *TNF* gene represents an opportunity to identify a potential moderator of the downregulation of circulatory levels of TNFα. In turn, the DNA methylation of the *TNF* gene has been explored with regard to noncommunicable disease risk factors.

Although research in this area is sparse, a handful of studies have investigated the links between noncommunicable disease risk and DNA methylation of CpG sites relating to expression of TNFα. In a study examining a student female population, lower methylation levels at the *TNF* promoter were associated with higher circulatory plasma levels of TNFα, and in turn were associated with higher levels of truncated fat (Hermsdoff et al., 2013). *TNF* methylation has also been negatively associated with dietary intake of folic acid, beta-carotene and cholesterol in an obese population (Bollati et al., 2014). However, the same study demonstrated blood levels of cholesterol were positively associated with *TNF* methylation, suggesting a complex relationship between dietary factors and *TNF*-related epigenetic processes. Intervention studies in obese populations have also implicated *TNF* methylation as a potential mechanism, with a baseline measure of *TNF* methylation predicted the responsiveness to a low-calorie diet (Campión,

Milagro, Goyenechea & Martínez, 2009; Cordero et al., 2011; Milagro et al., 2011). However, it should be noted that in these trials, the dietary intervention did not result in changes in *TNF* methylation. While firm conclusions cannot be drawn due to the limited volume of research in this area, there is evidence suggesting an association between higher methylation of the *TNF* promoter as a limiting risk factor for obesity, and circulatory plasma levels of *TNFα* may represent a pathway in this relationship.

To summarise, there is a growing volume of research that implies psychological experiences may play an important role in epigenetic processes (e.g., Cole, 2007; Miller et al., 2015). The importance of motivation in regulating goal-directed behaviour with implications for health and well-being has been highlighted (Ng et al., 2012). However, as yet there are no studies that have explored motivational processes as potential epigenetic mechanisms. Combined evidence suggests that methylation of *TNF* promoter may also have a positive influence on long-term health, and that healthy behaviour including diet and physical activity may be a potential limiting risk factor in this relationship. Therefore, investigating the association between motivational processes, healthy behaviour and *TNF* methylation represents a viable and scientifically appropriate endeavour. This is the central premise that forms the basis of the study presented in Chapter Five.

Summary and Overview of Thesis

There is a large volume of literature supporting the importance of high-quality motivational processes in adaptive human functioning. The primary aim of this thesis will be to extend this idea to biological and physiological markers implicated with improved health. Many motivational theories emphasizing the importance of high-quality motivation also highlight the implications for improved well-being (Ng et al., 2012). However, it is less well understood if the implications for improved well-being extend to physical health profiles. Furthermore, a key tenet of theories emphasizing the quality of motivation is the association with improved behavioral outcomes (Cerasoli et al., 2014). However, it is less clear is if there are motivational effects associated with physiological health that extend beyond that which is explained by engagement with the activity itself. For example, whether an individual exposed to high-quality motivation would benefit from improved performance and adaptive physiological profiles, or whether there is a psychophysiological cost associated with improved behavioral outcomes. Furthermore, motivational processes have been identified as key mechanisms in self-control and self-

regulation (Carver & Vargas, 2011; Hagger et al., 2010). However, debate surrounds the reliability of the effect of self-control exertion on multiple tasks, with motivational processes and physiological mechanisms are implicated as potential mechanisms (Baumeister & Vohs, 2016a). Given the associations between motivation, self-control and self-regulation, it seems prudent to explore behavioral outcomes through this empirical paradigm. Finally, motivational scientists have highlighted the importance of extending research into novel areas of biological science to strengthen key theoretical tenets (Di Domenico & Ryan, 2017). Furthermore, tentative steps have recently seen psychologists explore epigenetics as an important lens through which to explore human health and optimal human functioning (e.g., Cole, 2013). This thesis will therefore be the first empirical exploration of motivational processes and healthy behaviour as potential epigenetic mechanisms.

Study One (Chapter Two). Addressing the key aims of this thesis began by systematically reviewing the extant literature employing multidimensional motivational processes and salivary markers associated with activation of the endocrine system. The key aim of this review was to identify commonalities that exist between theoretical boundaries when examining physiological outcomes. Furthermore, specific attention was paid to assessing the quality of the extant research to address recent concerns that have been raised regarding examination of motivational process and physiological responses.

Study Two (Chapter Three). Based on the results of Study One, Study Two addressed some of the shortcomings within the extant literature. Adopting the sequential-task paradigm, this chapter explored whether the manipulation of autonomy-support would lead to adaptive cortisol profiles and improved self-control performance when compared with controlled regulation. Furthermore, the study explored whether these effects would persist over time.

Study Three (Chapter Four). Study Two provided further support for the value of high-quality motivation in attenuating the cortisol response. However, Study Two also contradicted the idea that high-quality motivation is facilitative for self-control performance. The aim of Chapter Four was therefore to replicate, improve and extend the study design employed in Chapter Three.

Study Four (Chapter Five). Chapter Two highlighted the instability of using endocrine-related responses within cross-sectional designs as a tool for assessing biomarkers that may have important long-term health implications. Study Four therefore explored a novel area for

motivational science, epigenetics, exploring the associations between high-quality motivation, healthy behaviour and the DNA methylation of the *TNF* gene.

General Discussion (Chapter Six). The final chapter of this thesis summarizes the novel key findings, discusses the emerging main themes, and identifies theoretical and practical implications. The limitations are also acknowledged, and ideas for future research and presented and discussed.

Chapter Two

Study One

The Relationship Between Motivation and Endocrine-Related Responses: A Systematic Review

Abstract

Multidimensional motivational theories differ in how they describe human motivation. Nonetheless, many are unequivocal in suggesting the type of motivation is as important as the quantity, with many theories emphasizing the association between the quality of motivation and enhanced well-being. A volume of literature has accumulated exploring how constructs contained within these theories relate to activation of the endocrine system. However, research is fragmented across several theories and determining the current state of the science is complicated. The aim of this paper is to systematically review the association between multidimensional motivational constructs and endocrine-related responses to determine which theories are commonly used, and what inferences that can be made. Web of Science, PubMed, PsycINFO and Scopus were searched for relevant literature. Inclusion required a quantitative measure of at least one endocrine-related response taken from a human subject, and a quantitative measure or experimental manipulation of a multidimensional motivational construct. The review identified 37 studies incorporating five distinct motivation theories and multiple endocrine-related responses. Across several theories, it was evidenced that high-quality motivation attenuated the cortisol response in evaluative environments. There was also evidence that motivational needs for power and affiliation were associated with lower and higher levels of salivary secretory immunoglobulin A, respectively. The need for power may play a role in increasing testosterone when winning a contest, however, this evidence was not conclusive. Overall, the emerging literature suggests differentiating between higher and lower quality motivation can aid understanding of physiological responses to psychological phenomena.

Keywords: Stress, HPA, hormone, immune functioning

Introduction

Motivation can be defined as the process invoking the energization and direction of behaviour towards positive stimuli (Elliot, 2006). There are many theories that explore motivation, yet a common feature of many contemporary models is the distinction between optimal and suboptimal types of motivation. Behaviour can be driven by motivational processes that simultaneously facilitate well-being, or activities can be founded on types of motivation that drive behaviour, but have deleterious consequences for well-being (e.g., Dweck, 2017; Elliot et al., 2011; Ryan & Deci, 2017). This idea can be investigated in numerous ways, but one method is to employ physiological measures of human functioning. By doing so, researchers can explore how psychological processes influence downstream physiological mechanisms implicated in health and well-being. Based on this idea, the aim of this paper is to systematically review the research relating to theoretically-derived motivational constructs and physiological responses observed in the endocrine system.

Traditionally, many definitions of motivation adopted a unidimensional perspective where the strength or magnitude of the energizing belief is the key determinant in driving behaviour. In other words, a greater quantity of motivation yields a greater likelihood of meeting personal goals and initiating behaviour (Elliot, 2006). Within this unidimensional paradigm, the link between the magnitude of motivation and physiological responses is not new (see, for example, Cannon, 1927). More recently, increased motivation for behavioural engagement has been associated with elevated aspects of cardiovascular functioning (Gendolla, 2017; Wright & Gendolla, 2012). However, this emphasis on the quantity of motivation differs to multidimensional classifications that not only convey the magnitude of motivation, but also the qualitatively different reasons why people are motivated to engage in goal-directed behaviour (McClelland, 1987; Ryan & Deci, 2017). For example, one might observe a student reading a book in a university library and determine that he or she is motivated to read; however, one would not be able to determine whether they are reading because they feel pressure due to an upcoming assessment, for personal development, or for pleasure. Several theoretical perspectives are unequivocal in suggesting that the type (i.e., quality) of motivation is as important as the quantity, if not more important. Furthermore, multidimensional perspectives provide implications for behaviour, growth, and well-being (Ryan & Deci, 2017). For example, intrinsic and extrinsic motivation have been shown to jointly predict performance, yet intrinsic motives

better predict the quality of performance (e.g., engaging in complex activities, such as tasks requiring creativity or attention to detail), while extrinsic motivation better predicts the quantity of performance (e.g., less complex tasks evaluated by counting discrete units of output; Cerasoli et al., 2014). More broadly, higher quality motivation is associated with improved outcomes across multiple contexts, including education, work, human development, exercise and sport (e.g., Deci & Ryan, 2014; Elliot et al., 2018; Roberts & Treasure, 2012; Ryan, 2012; Shah & Gardner, 2008). On the other hand, although lower quality motivation can still drive individuals to achieve behavioural outcomes, it may be accompanied by psychological dysregulation, such as increased stress, depressive symptoms, and clinical disorders (e.g., de Bruin, Bakker & Oudejans, 2009; Emery, Heath & Mills, 2016; Tuominen-Soini, Salmela-Aro & Niemivirta, 2008).

A volume of literature has accumulated that is concerned with how qualitatively different types of motivation relate to physiological responses, particularly focusing on activation of the endocrine system and the secretion of hormones to help maintain homeostasis. Hormones are responsible for the regulation of many activities including metabolism, immune functioning, reproductive processes and circadian rhythms (Black, 1994; Tortora & Derrickson, 2017). Activation of the endocrine system has adaptive advantages; for example, when confronted with a stressor, activation of the hypothalamic-pituitary-adrenal axis mobilizes the body's resources to meet a challenge or threat (Lupien, McEwen, Gunnar & Heim, 2009). However, the diversion of resources also has an immunosuppressive effect, which if persists can lead to mental and physical health dysregulation and the pathogenesis of disease (Glaser & Kiecolt-Glaser, 2005; McEwen & Stellar, 1993).

Motivational processes implicated in the pursuit of goals are assumed to play a key role in regulating the endocrine system, especially in response to a stressful situational threat to goal-directed behaviour (Carver & Scheier, 1999; Dickerson & Kemeny, 2004). For instance, when students were instructed to learn a new skill in a social-evaluative environment, those exposed to a motivational environment emphasizing normative performance experienced higher cortisol levels than those exposed to an environment with a greater emphasis on self-referenced learning (Hogue et al., 2017; Hogue et al., 2013). In a similar vein, students experienced higher cortisol levels when instructed to complete a puzzle-solving activity in a motivationally coercive manner, compared to a group instructed in an autonomy-supportive style (Reeve & Tseng, 2011). These

examples demonstrate that cortisol responses can be moderated by qualitatively distinct motivational emphases, however, this principle extends beyond cortisol. For example, the motivation to have control over others (i.e., the need for power; McClelland, 1987) has been explored in relation to several endocrine responses including testosterone, progesterone and estradiol (Stanton & Schultheiss, 2009). Broader indicators of immune function have also been used as biomarkers in psychophysiological research, particularly those related to stress, because they are theoretically and empirically linked with endocrine functioning. For instance, secretory immunoglobulin A (sIgA) is assumed to be modified by activation of the sympathetic-adrenal-medullary axis and hypothalamic–pituitary–ovarian axis; specifically, by cortisol, epinephrine and norepinephrine (Segerstrom & Miller, 2004). As such, sIgA is upregulated by acute laboratory stressors and downregulated by naturalistic acute (e.g., exams) and chronic stressors (Segerstrom & Miller, 2004). In a similar vein, the digestive enzyme salivary alpha-amylase (sAA) is also employed in psychoneuroendocrinology as a marker of sympathetic nervous system (SNS) activation induced by stress (Bosch, Ring, de Geus, Veerman & Amerongen, 2002; but see also Bosch, Veerman, de Geus & Proctor, 2011).

Despite the burgeoning volume of research concerning multidimensional motivation and endocrine-related responses, a review of the literature remains absent. Research is fragmented across several motivational theories, therefore, determining the current state of the literature and identifying avenues for future research is complicated. A trans-theoretical compilation will provide a comprehensive understanding of the relationships between multidimensional motivation and endocrine-related responses. In addition, the scientific approach taken to study the psychophysiology of motivation has come under criticism. In many instances, unwarranted conclusions are made and relationships between motivation and marker are overemphasized (Richter & Slade, 2017). Adopting a broad perspective to scrutinize the psychophysiological relationship between motivation and physiological response can illuminate the instances when these problems may have occurred. Therefore, the purpose of this study was to systematically review the literature on multidimensional motivational constructs and their associations with endocrine-related responses.

There are a variety of methods available for measuring endocrine-related responses including the use of plasma, urine and sweat. Although there are advantages and disadvantages to each of these methods of measurement, for social science research, saliva offers advantages

compared with alternative methods. Salivary responses present a valid, reliable and non-invasive method of reviewing acute and circadian patterns that limits the possibility of the method confounding the item of interest; for example, venipuncture can significantly increase cortisol levels (Smyth et al., 2013). Moreover, plasma collection requires specialist training, is time-consuming, expensive, and has ethical constraints (Kirschbaum & Hellhammer, 1994; Smyth et al., 2013). As such, the present article focused on salivary markers of endocrine-related responses[1].

Method

Reporting of this systematic review has adhered to the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) statement (Moher, Liberati, Tetzlaff, Altman, & The PRISMA Group, 2009).

Search Strategy

The databases used to search for relevant literature were Web of Science, PubMed, PsycINFO and Scopus. Unpublished theses and dissertations were searched using Proquest. The full-text and reference lists of extracted studies were also inspected for relevant literature. Search strategies were built around two groups of keywords: Motivation terminology and endocrine-related responses. A scoping search was carried out prior to the formal screening process that uncovered potential research in the following theories: self-determination theory (SDT; Ryan & Deci, 2017), achievement goal theory (AGT; Nicholls, 1984, 1989), needs theory (McClelland, 1987) and implicit theories (Dweck, 2016). Consequently, keywords related to these theories were included in the search strategy (see Appendix One). Motivational constructs were identified using the broad search term "motiv*", the names of specific theories (e.g., "self-determination"), or associated keywords (e.g., "nPower"). Examples of keywords used to identify endocrine-related responses included general terms (e.g.," *endocrin*", "hormon*"), and specific types of response (e.g., "testosterone", "cortisol")

Inclusion Criteria

Studies were required to (i) be published in the English language from 1970 up to and including May 2017; (ii) have a quantitative measure of at least one salivary endocrine-related response taken from human subjects of any age; and (iii) contain a theoretically-derived construct of multidimensional motivation; either measured quantitatively using validated questionnaire data, or an experimental manipulation of motivation. Unidimensional

conceptualizations of motivation were excluded (e.g., effort as a motivational indicator within motivational intensity theory; Brehm & Self, 1989; self-efficacy theory; Bandura, 1977[2]), along with atheoretical measures of motivation (e.g., studies that employed music as a motivational tool). No exclusion criteria were set in relation to participant characteristics or the study sample size.

Identification of Relevant Studies

Citation abstracts and full-text articles, together with screening questions were uploaded to an internet-based software program that facilitated systematic reviewing (Covidence, Veritas Health Innovation). Duplicates were automatically removed in the first instance by the software program, and in the second instance by the lead author and supervisor during the title and abstract screening.

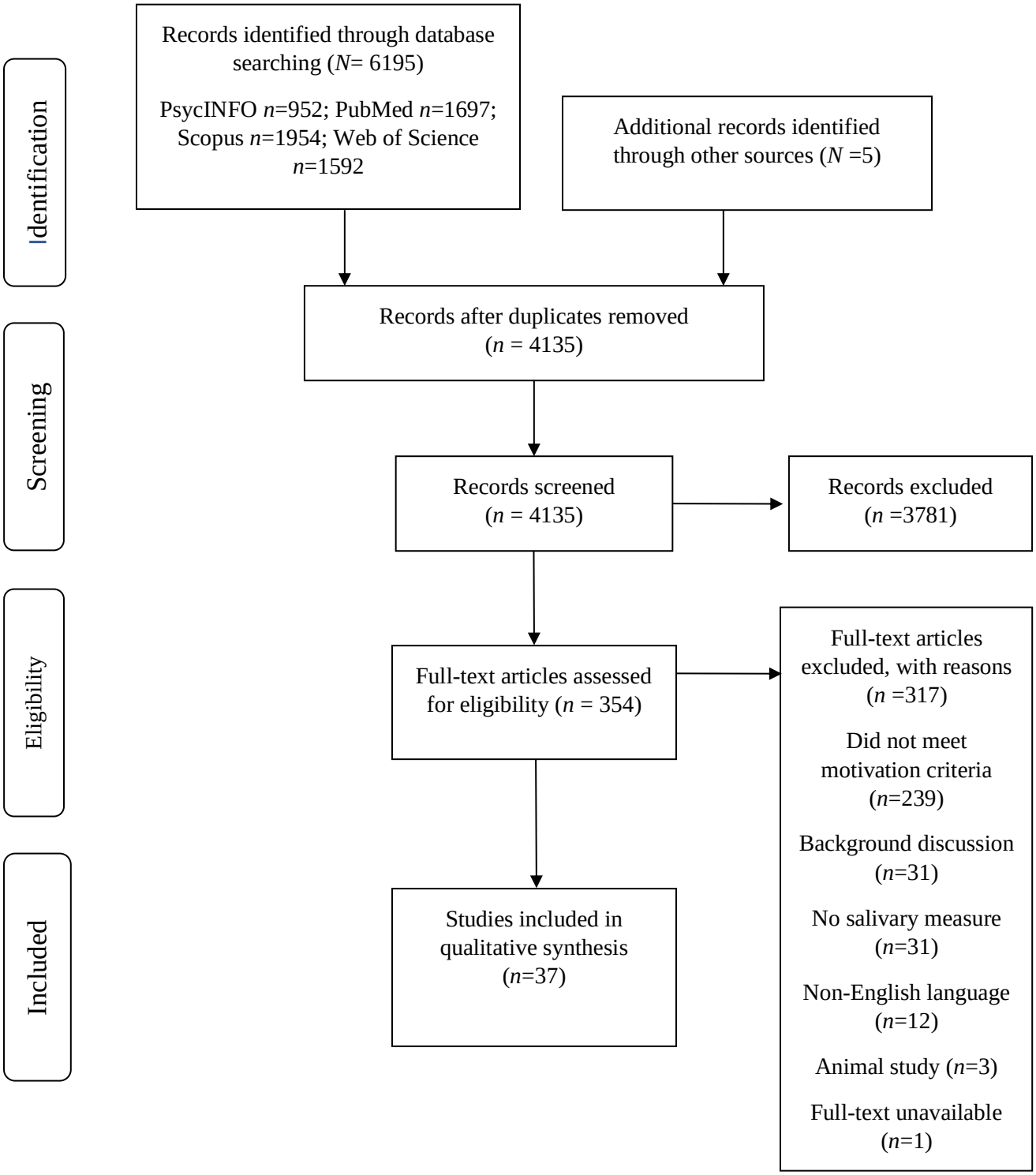
The lead author and PhD supervisor independently screened the results of the search strategy to determine whether the article met the inclusion criteria. All abstracts and titles were screened by both contributors. If abstracts were not available or did not contain sufficient information, the full-text was screened to determine potential eligibility. Upon completion of the title and abstract screening, both contributors subsequently examined the full-text of potential studies to determine whether it met the final inclusion criteria. Pre-determined protocol had a provision for disagreements over inclusion to be resolved through discussion and adjudication by an independent expert; however, no disagreement occurred. None of the contributors were blind to the journal titles, study authors, or institutions. The different phases of the process are summarized in Figure 2.1.

Data Extraction and Coding

To ensure consistency between lead author and supervisor, calibration exercises were conducted before starting the review. Using standardized forms, the two reviewers extracted data independently. The lead author completed the data extraction for all included studies and the supervisor randomly extracted data for ten studies to confirm accuracy. Data extracted included the underpinning motivational theory, endocrine-related response, participant demographic information, study methodology, and sample size. Due to the broad range of motivational constructs and the hazards of pooling data from diverse, non-randomized studies (Sterne, Egger & Moher, 2008), meta-analysis was deemed inappropriate for this review.

Figure 2.1

PRISMA flowchart information through the different phases of the systematic review



Observational and experimental studies were included in the review. A single measure of saliva indicated a cross-sectional study, with two or more collection timepoints treated as longitudinal data. Experimental studies included studies where the experimental manipulation related to the motivational theory (e.g., the manipulation of autonomous motivation in line with SDT). If the experimental manipulation was not motivation-based, but motivation was measured, the data extracted was classified as either cross-sectional or longitudinal. Primary experimental effects (e.g., analysis of variance statistics), longitudinal statistics inferring change (e.g., regression coefficients, controlling for baseline measures of the dependent variable), or correlational statistics (e.g., bivariate correlation) were extracted, depending on study design. A data extraction flowchart was constructed to assist identification and extraction of relevant statistics (Appendix Two).

Risk of Bias/Study Quality

Risk of bias was assessed using a modified version of the Downs and Black (1998) checklist. The original checklist comprised of 27 items measuring various aspects of quality assessment, however, 14 of these items were discarded due to lack of relevance (items were suited mainly to clinical trials). Of the remaining 13 items, three items were only relevant for experimental studies (e.g., blinding of participants). The scoring for each question followed the format of yes (1), and no or unable to determine (0), with a higher score representative of a low risk of bias (i.e., high quality).

Results

Study Characteristics

Thirty-seven published papers, incorporating 42 independent studies were included in the review. Most studies were experimental (n = 26), followed by longitudinal (n = 10) and cross-sectional designs (n = 6). Most studies utilized mixed-gender samples (n = 25) with three studies not reporting gender. Studies that employed men- (n = 9) or women-only (n = 5) samples did so to measure characteristics unique to that gender (e.g., medical contraceptive use, menstrual cycle) or physiological hormones that vary depending on gender (e.g., testosterone, progesterone, estradiol). In total, there were five motivational theories included in the extracted studies: Needs theory (McClelland, 1987, n = 30), self-determination theory (Ryan & Deci, 2017, n = 5), reversal theory (Apter, 2001, n = 3), achievement goal theory (Nicholls, 1984, n = 2) and implicit theory (Dweck, 2016, n = 2). Nine salivary hormonal or endocrine-related responses were

measured: Cortisol (*n* = 22), testosterone (*n* = 10), sIgA (*n* = 6), progesterone (*n* = 5), estradiol (*n* = 4), sAA (*n* = 2), epinephrine (*n* = 2), dehydroepiandrosterone (DHEA, *n* = 1) and norepinephrine (*n* = 1). When assessing risk of bias, most studies scored relatively highly (i.e., low risk of bias), with experimental studies scoring an average of 10.09 (out of 13), and observational studies scoring 7.57 (out of 10). The risk of bias for individual elements from the Downs and Black (1998) checklist is summarized in Table 2.1. Of note, however, was that the blinding of researchers (3 studies out of 23), reporting of exact *p*-values (21 studies out of 37) and reporting of power analysis (3 studies out of 37) are recommended practices that were relatively poorly observed across the reviewed papers.

Primary Results

A summary of all the key data extracted is presented in Table 2.2. This includes lead author, year of publication, study design, sample size, study features, study quality (risk of bias), motivational theory, endocrine-related response, motivational construct, direction of association and any supplementary comments. The following sections provide a description of each underpinning theory together with a commentary on the findings.

Self-determination theory. One cross-sectional study, one longitudinal study and three experimental studies used self-determination theory as a framework to examine motivational constructs and endocrine-related responses. Self-determination theory posits that humans flourish or are hindered according to the extent to which three basic psychological needs - competence, autonomy and relatedness – are satisfied (Ryan & Deci, 2017). Satisfaction of these psychological needs characterizes the degree to which motivation is well integrated with the sense of self; pursued autonomously and of high quality. On the other hand, low need satisfaction is the basis for low quality, controlled motivational regulation (Ryan & Deci, 2000a).

The cross-sectional study included satisfaction and thwarting of basic psychological needs, as well as coaching context variables, in a population of 120 junior athletes with sIgA the physiological response of interest. Basic psychological needs thwarting was positively associated with higher levels of sIgA, whereas psychological need satisfaction was unrelated (Bartholomew et al., 2011). In the longitudinal study, higher basic psychological need satisfaction was associated with lower cortisol measures before, during, and after performance of a ballet routine in a population of dancers (Quested et al., 2011). Two of the three experimental studies

Table 2.1.

Risk of Bias in Reviewed Studies (based on Downs and Black, 1998)

Item	Description	Frequency (max 37 unless Otherwise indicated)
1.	Is the hypothesis/aim/objective of the study clearly described?	34
2.	Are the main outcomes to be measured clearly described in the Introduction or Methods section?	37
3.	Are the characteristics of the participants included in the study clearly described?	29
4.*	Are the interventions of interest clearly described?	23/23
5.	Are the main findings of the study clearly described?	31
6.	Does the study provide estimates of the random variability in the data for the main outcomes?	31
7.	Have actual probability values been reported for the main outcomes except where the probability value is less than 0.001?	21
8. *	Was an attempt made to blind study subjects to the intervention they have received?	23/23
9. *	Was an attempt made to blind those measuring the main outcomes of the intervention?	3/23
10.	If any of the results of the study were based on data dredging, was this made clear?	35
11.	Were the statistical tests used to assess the main outcomes appropriate?	33
12.	Were the main outcome measures used accurate (valid and reliable)?	35
13.**	Did the study report the sample size required to detect a significant effect where the probability value for a difference being due to chance is less than 5%?	3

* Items 4, 8 and 9 relate only to the 23 experimental studies.

** The wording of this item was amended slightly to asses reporting of power analysis, rather than whether the study had sufficient power, with studies scored yes (1) and no (0).

Table 2.2

Summary of Key Study Characteristics of Reviewed Studies

Author, year	Study design[a]	Sample size (F)	Study features (M_{age})	Quality score	Theory	Endocrine response (number of data points)	Motivational construct (significance/direction of relationship)	Comments
Bartholomew et al., 2011	3	120 (92)	Junior athletes (14.51)	8/10	SDT	sIgA (1)	Need satisfaction (ns) Need thwarting (+)	
Cuevas et al., 2014	2	94(94)	Breast cancer survivors (56.2)	8/10	RT	C (10)	Telic (ns) Conformist (ns) Mastery (ns) Autistic (ns)	
Dabbs et al., 1990	3	110(53)	College students (nr)	6/10	NT	T (1)	nPower (ns)	
Duffy et al., 2017	1	106(106)	Female (21.2) Social acceptance v rejection	9/13	NT	C (3), P (3)	nAff (ns)	Study design was experimental; however, the manipulation was not related to motivation, so correlations only are reported.
Edelstein et al., 2010	3	102(44)	Undergraduate students (18.79)	8/10	NT	E (1)	nAff (ns)	
Filaire et al., 2007	2	10(0)	Elite paragliders (27.2)	7/10	RT	C (6)	Serious mindedness (ns) Planning orientation (ns) Arousal avoidance (ns)	The only significant finding across six timepoints was association with serious mindedness at t4 (r = -0.73).
Hogue et al., 2013	1	107 (61)	University students (19.89). Juggling task: Task/ego	10/13	AGT	C (7)	Ego (+) Task (-)	Significant climate × time interaction. Cortisol response increased in the ego condition compared with the task condition.
Hogue et al., 2017	1	47 (26)	Middle school students (11.98). Juggling task: Task v ego	11/13	AGT	C (4)	Ego (+) Task (-)	Significant climate x time interaction. Cortisol response increased in the ego condition compared with the task condition.
Jemmott et al., 1983	2	64(16)	Dental students (23.4) 10-month longitudinal	7/10	NT	sIgA (5)	nAff (+) nPower (-)	The relationship between nAff and sIgA was higher than the nPower-sIgA relationship across all timepoints.

Author, year	Study design[a]	Sample size (F)	Study features (M_{age})	Quality score	Theory	Endocrine response (number of data points)	Motivational construct (significance/direction of relationship)	Comments
McClelland et al., 1982	3	133(0)	Male prisoners (28.5)	8/10	NT	sIgA (1)	nPower (nr) nAff (nr)	The direct relationships independent of stress were not reported.
McClelland et al., 1980	2	27(0)	College sophomores (nr)	6/10	NT	sIgA (2)	nPower (nr) nAff (nr)	The direct relationships independent of stress were not reported.
McClelland et al., 1985	2	46(17)	College students(nr) Examination stress	7/10	NT	sIgA, NE (3)	IgA: nPower (-), nAff (ns) NE: nPower (+); nAff (ns)	sIgA: nPower dominant participants experienced a reduction in sIgA 105mins post exam. This difference was significant compared with baseline and compared with nAff dominant participants. NE: Increased in nPower dominant participants post-exam.
McClelland et al., 1987	1	61(30)	University students (19.0) Emotional arousal film: nPower v nAff	6/13	NT	C, EP, NE (2)	nPower (ns) nAff (ns)	
McClelland & Kirshnit, 1988	1	132(nr)	College students (nr) Emotional arousal film: nPower v nAff	9/13	NT	sIgA (3)	nPower (ns) nAff (+)	In the nAff condition, there was evidence of significant changes in sIgA post-film
Quested et al., 2011	2	61(41)	Ballet dancers (19.3) Challenge v threat appraisal	9/10	SDT	C (5)	BPNS (-)	BPNS was not significant when challenge appraisals were included in the model.
Reeve & Tseng, 2011	1	78(53)	Undergraduates (nr) Puzzle solving: Autonomous v controlled v control group	9/13	SDT	C (3)	Autonomous (-) Controlled (+)	

Author, year	Study design[a]	Sample size (F)	Study features (M_{age})	Quality score	Theory	Endocrine response (number of data points)	Motivational construct (significance/direction of relationship)	Comments
Schultheiss et al., 1999	1	42(nr)	University students (20.26) Contest outcome: Win v loss	8/13	NT	T (3)	pPower win (+) loss(ns) p+sPower win (-) loss(ns)	pPower refers to participants for whom sPower was absent; p+sPower refers to participants high in pPower who also exhibited sPower.
Schultheiss & Rohde, 2002	1	66(0)	Vocational college students (23.83) Contest outcome: Win v loss	9/13	NT	T (6)	nPower win (nr) loss (nr)	Direct effect of condition, nPower and testosterone not reported. There was a significant interaction between inhibition, nPower and testosterone at t5 in winners, with no significant effect among losers.
Schultheiss et al., 2014 (study 1)	2	108(53)	University students (20)	10/13	NT	C (2)	nAch (-)	Re-analysis of Wirth et al. (2006) results, excluding win/loss manipulation.
Schultheiss et al., 2014 (study 2)	1	62(31)	University students (23.87) TSST v Control	-	NT	C (2)	nAch (-)	nAch predicted a reduced cortisol response to TSST, but not to a control task.
Schultheiss et al., 2003	2	54(18)	University students (nr)	7/10	NT	T, P, E (3)	nAff (nr) nPower (nr)	The study examined contextual effects related to menstrual cycle and relationship status. Direct relationships independent of these variables were not reported.
Schultheiss et al., 2012	3	92(50)	University students (23)	9/10	NT	C, P (1)	nPower (nr) nAff (nr) nAch (nr)	Direct associations were not reported, only motivational incongruence which is the difference between explicit and implicit motivation.
Schultheiss et al., 2004	1	60(39)	Undergraduate students (19.78). Emotional arousal film: nAff v nPower v control	10/13	NT	P, T (3)	nAff (ns) nPower (ns)	Main effect for experimental condition not significant. Post-hoc analysis offered some support for the hypothesis.

Author, year	Study design[a]	Sample size (F)	Study features (M_{age})	Quality score	Theory	Endocrine response (number of data points)	Motivational construct (significance/direction of relationship)	Comments
Schultheiss et al., 2005 (study 1)	1	95(0)	Undergraduate students (19.67) Contest outcome: Win v loss	10/13	NT	T (6)	nPower win (ns) nPower loss (ns)	Main effect for experimental condition was not significant. There was a significant condition × testosterone effect at t5 between conditions.
Schultheiss et al., 2005 (study 2)	1	75(75)	Undergraduate students (20.82) Contest outcome: Win v loss	-	NT	T (6)	nPower win (ns) nPower loss (ns)	Main effect for experimental condition was not significant. There was a significant condition × testosterone effect at t4 between conditions.
Sieber et al., 2016	1	69(34)	Middle school children (14.16) Autonomy supportive v autonomy restrictive v control.	11/13	SDT	sAA (3)	Autonomy supportive (ns) Autonomy restrict (+) Control group (+)	Participants with a high implicit autonomy disposition displayed lower sAA responses when exposed to autonomy-supportive vignettes, when compared to when they were exposed to controlling or neutral teaching styles. The opposite pattern was found in students with a low implicit autonomy disposition.
Slatcher et al., 2011	3	74(0)	Psychology undergraduates (nr)	9/10	NT	T (1)	nPower (ns)	
Sørensen et al., 2015	1	97(85)	Healthcare professionals (44.5, 42.5)	13/13	SDT	C (4)	IMI (nr)	The study measured both cortisol and the IMI; however, the two measures were DVs. The relationship between the two was not an aim of the study and was not reported.
Stanton & Edelstein, 2009	2[b]	40(40)	University students (18.58)	11/13	NT	E (2)	nPower (+)	
Stanton & Schultheiss, 2007	1	49(49)	University students (19.96). Contest outcome: Win v loss	10/13	NT	T, E (6)	T: nPower win(ns) loss(ns) E: nPower win (+) loss(ns)	Data drawn from Wirth et al., 2006, study two. Estradiol: nPower interaction was significant at aggregated t4, t5 & t6 in winners.

Author, year	Study design[a]	Sample size (F)	Study features (M_{age})	Quality score	Theory	Endocrine response (number of data points)	Motivational construct (significance/direction of relationship)	Comments
Thatcher et al., 2003	2	23(1)	Skydivers (nr)	7/10	RT	C (2)	Telic-Paratelic (ns) Negativistic – conformist (ns) Arousal seeking - arousal avoidance (ns) Meta-motivational dominance (ns)	
Vongas et al., 2017 (Study 1)	1	84(0)	Undergraduate students (22.01) Contest outcome: Win v loss	11/13	NT	T (3)	pPower win (-) loss(ns)	pPower moderated the relationship between competition outcome and testosterone change. Increased pPower in winners attenuated the decrease in testosterone.
Vongas et al., 2017 (Study 2)	1	72(0)	Undergraduate students (21.39) Contest outcome: Win v loss	--	NT	T (3)	pPower win (-) loss(ns)	pPower moderated the relationship between competition outcome and testosterone change. Increased pPower in winners attenuated the decrease in testosterone.
Wegner et al., 2014	1	59(32)	High school students (14.8) Task: Physical v psychosocial v control	11/13	NT	C (2)	nAff: Psychosocial (-) Physical (ns) Control (ns)	The psychosocial task is the most appropriate measure as it is unlikely the physical task (15 mins moderate running) or control would be sufficient to provoke a cortisol response.
Wegner et al., 2015	1	57(33)	High school students (14.8) Task: Physical v psychosocial v control	11/13	NT	C (2)	nPower: Psychosocial (+) Physical (ns) Control (-)	The psychosocial task is the most appropriate measure as it is unlikely the physical task (15 mins moderate running) or control would be sufficient to provoke a cortisol response.
Wiemers et al., 2015	1	72 (34)	University sample (nr) TSST v control	10/13	NT	C, sAA (4)	C × nPower (-) C × nAch (ns) C × nAff (ns) sAA × nPower (+) sAA × nAch (ns) sAA × nAff (ns)	

Author, year	Study design[a]	Sample size (F)	Study features (M_{age})	Quality score	Theory	Endocrine response (number of data points)	Motivational construct (significance/direction of relationship)	Comments
Wirth & Schultheiss, 2006	1	87(38)	Undergraduate sample (19.7) Emotional arousal film: HoC v FoR v control	10/13	NT	C, P (3)	C × nPower (ns) C × nAff (+) C × nAch (ns) P × nPower (ns) P × nAff (+) P × nAch (ns)	Reported associations are between hormone and implicit motive relationships in women only independent of experimental condition. Furthermore, nAff positively predicted post-film progesterone in the FoR condition.
Wirth et al., 2006 (Study 1)	1	66(0)	Vocational college (23.8) Contest outcome: Win v loss	9/13	NT	C (6)	nPower win (ns) loss (+)	Implicit power motivation predicted cortisol increases in losers, but not in winners of the contest.
Wirth et al., 2006 (Study 2)	1	108(53)	University students (20.3) Contest outcome: Win v loss	-	NT	C (6)	nPower win (ns) loss (ns)	Cortisol measurement aggregated into pre-test (t1-t3) and post-test (t4-t6) scores. nPower positively predicted cortisol response in male losers, but not in winners. Post hoc analysis revealed evidence of an effect in participants tested after 2pm.
Yang et al., 2015	1	50(26)	University students (M=19.9; F=18.8) D2 test of attention: positive v negative v no feedback.	11/13	NT	C (2)	nAch (ns)	Study was experimental; however, the manipulation was not related to motivation. Residualizsed cortisol was significantly associated with the positive feedback condition.
Yeager et al., 2015 (Study 1)	1	60(27)	High school students (15.61) Learning task: Incremental v control	13/13	IT	C (2)	Incremental (-)	Cortisol declined significantly in the implicit theory incremental intervention compared with control group.
Yeager et al., 2015 (Study 2)	1	205(nr)	High school students (nr) Learning task: Incremental v control	-	IT	C, DHEA (6)	Incremental (ns)	Overall intervention effect was absent, however a significant attenuated cortisol and DHEA response was observed between conditions on days 8 and 9.

Notes. F = female; SDT = self-determination theory; sIgA = salivary secretory immunoglobulin A; ns = non-significant; (+) = positive association; RT = reversal theory; C = cortisol; nr = not reported; NT = needs theory; T = testosterone; P = progesterone; nPower = need for power; nAff = need for affiliation; t = timepoint; E = estradiol; AGT = achievement goal theory; (-) = negative association; NE = norepinephrine; EP = epinephrine; BPNS = basic psychological needs satisfaction; TSST = Trier social stress test; nAch = need for achievement; sAA = salivary alpha-amylase; IMI = intrinsic motivation inventory; HoC = hope of closeness; FoR = fear of rejection; M = male; IT = implicit theory; DHEA = dehydroepiandrosterone.

[a]Study design: 1 = experimental; 2 = longitudinal; 3 = cross-sectional.

[b]Stanton and Edelstein (2009) took saliva measures at two timepoints, however they were within an hour or each other and the two measures were collapsed for analysis with a correlation reported between estradiol and nPower.

manipulated motivation as the independent variable. During a puzzle solving task, a decline in cortisol in a condition supporting autonomous motivational regulation, an increase in cortisol in a condition fostering controlling motivational regulation, and no change in a neutral condition was observed (Reeve & Tseng, 2011). A further study investigated the moderating effect of implicit autonomous disposition on the sAA response to different motivation inducing environments (Sieber, Schüler & Wegner, 2016). In a motivationally controlling environment, an increase in sAA was observed among those who displayed a high autonomous disposition, and a decreased sAA response was observed in participants with low autonomy disposition, with this pattern also observed in the control group. Conversely, in the autonomy supportive condition, those with a high autonomous disposition experienced a lower sAA response, while those with a lower autonomy disposition experienced a higher sAA response. A third experimental intervention involved obstetric anaesthesia training and was not related to motivation (Sørensen et al., 2015). Intrinsic motivation and cortisol were measured as dependent variables, however the direct relationship between the two was not reported.

Reversal theory. Three longitudinal studies investigated reversal theory (Apter, 2001), with cortisol the outcome of interest. Reversal theory posits that individuals are dynamically motivated to be either telic (serious) or paratelic (playful). Motivation was measured using the Apter Motivational Styles Profile (Apter, Mallows & Williams, 1998), and examined metamotivational dominance, which is calculated by subtracting telic from paratelic scores. Two studies revealed no significant associations between motivational states and cortisol in skydivers (Thatcher, Reeves, Dorling, & Palmer, 2003) or breast cancer survivors (Cuevas et al., 2014). A third study reported one significant relationship; a negative association between the telic subscale of serious mindedness and cortisol 10 mins before a paragliding flight (Filaire, Rouveix, Alix, & Le Scanff, 2007).

Achievement goal theory. Two studies measured cortisol concurrently with learning how to juggle in either a task- or ego-oriented environment (Nicholls, 1989). A task-oriented motivational environment fosters cooperation, values high effort and encourages personal development. On the other hand, an ego-oriented motivational environment promotes competition between group members, punishes mistakes and rewards high performance (Nicholls, 1984). The two studies were conducted by the same lab, employing near identical methods; one with university students (Hogue et al., 2013) and one with middle-school students

(Hogue et al., 2017). The results of both studies revealed a significant time by environment interaction, with the ego-oriented environment inducing significantly higher cortisol levels at 15- and 30-minutes post-intervention, relative to the task-oriented environment.

Implicit theories. One research paper encompassing two experiments was grounded in implicit theories (Dweck, 2016). Individuals who hold incremental implicit beliefs consider intelligence and other traits to be malleable, and their goal is to increase their competence. Conversely, individuals holding entity beliefs consider traits as fixed, and their goal is to seek positive judgements (Dweck, 2016). The studies examined implicit theories in relation to cortisol (study 1 & 2) and DHEA (study 2) in a population of high-school students (Yeager et al., 2016). Study one was laboratory-based with participants randomly assigned to either an incremental theory or active control reading and writing exercise, followed by the Trier social stress test (TSST; Kirschbaum, Pirke, & Hellhammer, 1993). The incremental group experienced a reduction in cortisol, and the control group experienced an increase in cortisol. Study two was a pre-registered double-blind intervention with the same experimental protocol implemented among a larger pool of participants with saliva collected over a 9-day period. Although an overall intervention effect was absent, an attenuated cortisol and DHEA response in the incremental belief condition (compared to the control condition) was observed on the final two days (days 8 & 9) of the intervention.

Needs theory. Thirty studies employed methods designed to measure or manipulate implicit needs for achievement (nAch), power (nPower) or affiliation (nAff). nAch is the desire to accomplish challenging goals, while avoiding goals that are easy to achieve or excessively difficult (McClelland et al., 1953). nPower is the desire to control other people to accomplish goals that are usually related to status or success (McClelland, 1975). nAff is the desire to achieve a sense of belonging and achievement via collaboration (Koestner & McClelland, 1992). These needs are usually measured implicitly, although they can also be represented explicitly (McClelland et al., 1989). Most of the studies included in the review focused on one or two of these needs.

Of the six cross-sectional studies, one study demonstrated a significant positive association between nPower and estradiol (Stanton & Edelstein, 2009). Three studies reported non-significant associations between nPower and cortisol (Dabbs Jr, Hopper, & Jurkovic, 1990); nPower and testosterone (Slatcher, Mehta & Josephs, 2011); and nAff and estradiol (Edelstein,

Stanton, Henderson & Sanders, 2010), respectively. Two studies did not report the associations between implicit needs and sIgA (McClelland, Alexander & Marks, 1982), cortisol or progesterone (Schultheiss, Patalakh & Rösch, 2012).

Most of the experimental and longitudinal studies investigating needs theory were undertaken by the same group of researchers and measured multiple motivational constructs and endocrine-related responses. The program of research began with evidence indicating that seven college students who had a high nPower, inhibition and power stress profile exhibited lower levels of sIgA, relative to a comparison group of 19 participants; however, the isolated relationship between nPower and sIgA was not reported (McClelland, Floor, Davidson & Saron, 1980). A subsequent longitudinal study revealed that dental students who were nPower dominant (high in nPower, low in nAff) experienced significantly lower sIgA levels over a ten-month period when compared with students who were nAff dominant (high in nAff, low in nPower); especially during the summer examination period (Jemmott et al., 1983). In a further longitudinal study undertaken during an exam, students who were nPower dominant (versus nAff dominant) experienced significantly reduced levels of sIgA 105 mins post-exam. However, there was no significant difference immediately after the exam, or differences in epinephrine between groups (McClelland et al., 1985). Finally, Schultheiss, Dargel and Rohde (2003) observed nPower and nAff along with testosterone, progesterone and estradiol at three-time points. Direct effects independent of relationship status and gender were not reported. However, when averaged across the timepoints, nPower was positively correlated with testosterone in men and single women; positively correlated with estradiol in engaged women; and nAff was negatively correlated with progesterone in men.

Further research experimentally explored needs theory by using excerpts of films designed to arouse implicit needs. Examples of films used include *Bridges of Madison County* (Eastwood, 1995; arousal of nAff) or *The Godfather II* (Coppola, 1974; arousal of nPower). The first study revealed no associations among nPower and nAff and norepinephrine, epinephrine and cortisol (McClelland, Patel, Stier & Brown, 1987, study 1). In a subsequent study, a film presented to arouse nAff led to a corresponding increase in sIgA after the film, however, a film presented to arouse nPower had no effect on sIgA (McClelland & Kirshnit, 1988). In a third study, films arousing nPower or nAff had no effect on testosterone or progesterone (Schultheiss, Wirth & Stanton, 2004), despite post-hoc analysis suggesting nuanced relationships. A final

study used two films to arouse either a hope of closeness (approach affiliation) or fear of rejection (avoidance affiliation) experimental conditions (Wirth & Schultheiss, 2006). nPower, nAff and nAch were measured pre- and post-task, with cortisol and progesterone the outcome of interest. Non-significant correlations were reported between nPower and nAch and endocrine-related responses in women, and any motive and endocrine-related response in men. Post-hoc analysis of the female sample revealed baseline nAff predicted post-film increases in progesterone in the fear of rejection condition. Furthermore, there were positive relationships between baseline nAff and cortisol, and post-film nAff and progesterone in women across all timepoints, irrespective of experimental conditions.

Several studies have considered the relationship between motivational implicit needs and endocrine response during a variety of social-evaluative tasks. Reanalysis of data from an earlier study (Wirth, Welsh & Schultheiss, 2006) demonstrated that higher nAch predicted lower cortisol response during a visuomotor competition, irrespective of competitive outcome (Schultheiss, Wiemers & Wolf, 2014, study 1). This association was replicated in response to the TSST (relative to a control condition; Schultheiss et al., 2014, study 2). A third study found TSST-induced nPower predicted a greater increase in sAA and lower cortisol response, relative to a friendly version of the TSST that did not induce nPower (Wiemers, Schultheiss & Wolf, 2015). Two further social-evaluative tasks examined nAff and nPower in relation to cortisol change in high school students across three experimental conditions; physical stress, psychosocial stress and a control task. In the first study, higher nAff negatively predicted cortisol change, and this was largely attributable to participants in the psychosocial stress condition, rather than the physical stress and control conditions (Wegner, Schüler & Budde, 2014). The same experimental design was used in a follow-up study examining nPower and cortisol (Wegner, Schüler, Scheuermann, Machado & Budde, 2015). nPower was associated with a greater cortisol response in the psychosocial stress group, a lower cortisol response in the control condition and no observed change in the physical stress condition. In a further study employing a challenging social-evaluative cognitive task with positive, negative or neutral bogus feedback, nAch did not predict cortisol response overall, however nAch dampened the cortisol response in the negative feedback condition (Yang, Ramsey, Schultheiss & Pang, 2015). Most recently, nAff was explicitly measured as a predictor of cortisol and progesterone in a task that manipulated

social acceptance or rejection. Explicit nAff was not associated with cortisol or progesterone at any of the timepoints (Duffy, Harris, Chartrand & Stanton, 2017).

Six studies examined individual differences in nPower and endocrine responses in the context of winning or losing a contest, often examining males and females separately due to known hormonal differences. The contest pits two participants in an artificial competition against each other on a cognitive task, with task difficulty manipulated so the pre-designated winner has a significant advantage. The first study compared pPower (a relatively personalized need for power) to sPower (a relatively altruistic, socialized need for power; Schultheiss, Campbell & McClelland, 1999). Whether winning a task or simply imagining winning, participants high in pPower but for whom sPower was absent experienced a significant increase in testosterone. Participants high in pPower and for whom sPower was present demonstrated no change in testosterone after imagining success, and a significant negative testosterone response to winning the task. No association between either type of need for power and testosterone was observed in participants who lost the task. Two further studies demonstrated very few significant relationships, and those that were reported were nuanced. In the first study, employing an all-male sample, nPower predicted increased testosterone among winners who were low in activity inhibition (i.e., the frequency of the use of the word "not" in the Picture Story Exercise, a tool primarily used to assess implicit needs) at the fifth of six measurement points (Schultheiss & Rohde, 2002). In a second study, males and females were tested separately. In the male sample, a significant negative correlation between nPower and testosterone among losers, and a marginally significant positive correlation among winners was observed at the fifth of six timepoints (Schultheiss et al., 2005, study 1). In the female sample, a significant positive nPower and testosterone association in losers at the forth of six timepoints was observed, with no significant association observed among winners (Schultheiss et al., 2005, study 2). Most recently, Vongas and Al Hajj (2017) conducted two studies using a male sample, collecting testosterone at three timepoints. Across both studies, winners' testosterone levels decreased significantly, however higher pPower in winners resulted in a relatively smaller decrease in testosterone, compared to low pPower. No significant effects between pPower and testosterone were observed in losers.

Finally, two win/loss studies examined cortisol and estradiol as endocrine responses. In an all-male sample, nPower positively predicted cortisol response in losers, but not in winners (Wirth et al., 2006, study 1). In a mixed-gender sample, only a negative trend (p = .08) was

observed between nPower and cortisol in winners tested after 2:00pm, but not losers (Wirth et al., 2006, study 2). Further analysis was performed on Wirth and colleagues mixed-gender sample by extracting estradiol in the female participants only (Stanton & Schultheiss, 2007). nPower had a positive association with estradiol among winners, but not losers.

Discussion

The aim of this systematic review was to investigate multidimensional motivational theories and constructs, and their associations with salivary endocrine-related responses. Comparing across theoretical boundaries facilitates understanding. It is possible to explore whether the psychophysiological processes are congruent with the broad tenets of the respective theory and whether there are cross-theory trends regarding each endocrine-related response. A further goal of the review was to evaluate the quality of the research and consider the methodological validity and conclusions made (see Richter & Slade, 2017). These goals are discussed in the sections below.

Cortisol was the most frequently studied hormonal response, and changes as a function of the quality of motivation induced were consistently observed. Task-oriented goal involvement, support for autonomous motivational regulation, and incremental implicit theories are hypothesized to be high quality motivational bases within their theoretical umbrella (AGT, SDT and implicit theories, respectively). These higher quality motives attenuated cortisol secretion in social-evaluative learning tasks, when compared to lower quality motivational foundations (Hogue et al., 2017; Hogue et al., 2013; Reeve & Tseng, 2011; Yeager et al., 2016, study 1). In all the studies, the effect sizes were large, risk of bias was relatively low, and the broad findings were replicated in a pre-registered study with excellent methodological rigor (Yeager et al., 2016, study 2). The fundamental ingredients of high-quality autonomous motivation (i.e., basic need satisfaction) were also linked to reduced cortisol levels in a field-based observational study (Quested et al., 2011). The qualitative nature of motives and the subsequent physiological impact identified in this review complement other recent biological links to multidimensional theories of motivation (e.g., Di Domenico & Ryan, 2017). This line of enquiry suggests that biological processes associated with improved human functioning should be integrated with motivational and psychological theory to enhance the validity and improve understanding of the mechanisms underpinning these processes (Ryan & Deci, 2017).

In contrast to theories that explicitly focus on different qualities of motivation (e.g., AGT, SDT, and implicit theories), theories that simply differentiate types of motivation revealed less conclusive findings relating to cortisol. For example, nPower exacerbated cortisol response in one psychosocial stress condition (Wegner et al., 2015); however, a lower cortisol response was observed in a nPower-inducing TSST, relative to a friendly TSST (Wiemers et al., 2015). The link between nAff and cortisol was also equivocal, with positive (Wirth & Schultheiss, 2006), negative (Wegner et al., 2014) and non-significant (Duffy et al., 2017; McClelland et al., 1987) associations reported. nAch did provide a more consistent relationship, predicting a dampened cortisol response during visuomotor competition, the TSST, and in response to negative feedback (Schultheiss et al., 2014 study 1 & 2; Yang et al., 2015). However, there were also experimental studies where this relationship was not observed (Wiemers et al., 2015; Wirth & Schultheiss, 2006). Finally, studies grounded in reversal theory (Apter, 2001) did not evidence their hypotheses. Telic or paratelic metamotivational states did not demonstrate any consistent relationship with cortisol (Cuevas et al., 2014; Filaire et al., 2007; Thatcher et al., 2003).

In summary, in a variety of situations, higher quality motivation was associated with an attenuated cortisol response and lower quality motivation was associated with an increased cortisol response, thus demonstrating a concomitant relationship (Cacioppo & Tassinary, 1990). This finding has two potential implications. On the one hand, poor quality motivation can be viewed as provoking an adaptive physiological response aimed at mobilizing resources to help the individual manage the stress of social-evaluation (e.g., Carver & Vargas, 2011). This conclusion, however, is overly simplistic. Despite the short-term benefits bestowed by cortisol release, acute cortisol responses are implicated with suppression of immune functioning (Segerstrom & Miller, 2004). Furthermore, frequent over-activation of the HPA axis (viz. allostatic load, McEwen, 1998) is implicated with increased risk for disease and dysregulated mental health (Lupien et al., 2009; McEwen & Stellar, 1993). Higher quality motivation typically deemphasizes normative evaluation in favour of self-referenced improvement (Murayama, Elliot & Friedman, 2012). Therefore, the more likely implication of the present review is high quality motivation may alleviate the perception of threat often associated with social evaluation, thus lowering physiological stress-related responses and facilitating long-term optimal functioning. The association between high versus low quality motivation and cortisol response may have implications for performance on complex and simple tasks. It is conceivable

that poor quality motivation is better for simple performance requirements because the heightened cortisol response does not impede, or even facilitates, basic (e.g., repetitive) performance. On the other hand, the attenuated cortisol response associated with high quality motivation is required for successful performance and engagement in complex tasks (see Cerasoli et al., 2014). Investigating these motivational processes would add a new dimension to the present investigation.

Six studies; five based on needs theory, and one based on SDT, examined the relationship between motivation-related constructs and sIgA. The main finding in the needs theory-based work was that nAff, relative to nPower, was longitudinally and experimentally associated with an enhanced sIgA response. This association was found during times of acute stress (i.e., exam periods; McClelland et al., 1985), in response to need-provoking films (McClelland & Kirshnit, 1988), and over a ten-month academic calendar (Jemmott et al., 1983). sIgA represents a complex indicator of human functioning (Bosch et al., 2002). Nonetheless, immunoglobulins combat bacteria and viruses, as well as triggering immune processes to target infection (Moser & Leo, 2010), and are therefore an indicator of heightened acute immunological functioning (Brandtzaeg, 2003). As such, nAff, relative to nPower, seems to be associated with immunoenhancing effects. Indeed, nPower is associated with greater self-reported illness (McClelland et al., 1980). Assuming that nAff leads to some degree of satisfaction of that need, this trend aligns with the large volume of literature documenting the physiological health benefits of social support networks (See Taylor, 2007; Kemeny, 2009 for reviews). Although needs theory does not emphasize the quality of motivation as much as other theories considered in this review, this conclusion may imply that there should be such a qualitative distinction between nAff and nPower, at least with regard to immune function.

The SDT-based study examining sIgA as an outcome demonstrated that psychological needs thwarting is associated with an enhanced immune response (i.e., higher sIgA; Bartholomew et al., 2011), seemingly contradicting a key tenet of SDT. However, this observation was cross-sectional and, along with several needs theory based cross-sectional work, may reflect a broad methodological weakness, rather than a theoretical nuance. Endocrine-related responses are dynamic in nature and are sensitive to moment-to-moment variation. For example, cortisol is characterized by diurnal variation, is sensitive to caffeine, smoking, pregnancy, illness and medication (Kirschbaum & Hellhammer, 1994; Smyth et al., 2013). Acute

negative events are typified by an increased endocrine-related response, while chronic negative events are often, but not always, characterized by a flattened response (e.g., Miller et al., 2007; Taylor, Turner, Gleeson, & Hough, 2015). Many other hormones are sensitive to variation, for example, progesterone and estradiol during the menstrual cycle (Tortora & Derrickson, 2017), and sIgA is modulated by stress reactivity (Bosch et al., 2002). In conclusion, measuring endocrine-related responses at a single timepoint is fraught with physiological and methodological pitfalls and based on the present findings should be avoided.

The second theme that emerged from the needs theory-based work is that winning a contest generally provoked increases in both testosterone and estradiol in participants high in nPower. However, this response did not occur to the same extent in winners who exhibited a more socialized form of nPower, where dominance is attained through prosocial behaviour. In losing situations, there was often a non-significant relationship between nPower and endocrine-related response. In other words, winning is positively associated with dominance-related hormonal responses in individuals who have a strong desire to demonstrate dominance. Testosterone is frequently associated with dominant behaviour, as is estradiol in women to a lesser extent (Stanton & Schultheiss, 2007). For individuals who thrive on situations where they can display dominance, winning, as opposed to losing, a contest bestows psychological and physiological benefits that prepare them for future dominance-related opportunities (Mazur & Booth, 1998). Thus, the associations between nPower and testosterone in competitive situations map well on to existing models of dominance behaviour, implicating nPower as a potentially important motivational process within this relationship.

The remaining endocrine-related responses investigated in relation to needs theory revealed mixed findings. The only significant association observed for progesterone was with nAff (Wirth & Schultheiss, 2006); however, this was outweighed by non-significant associations with nAff (Duffy et al., 2017), nAff and nPower (Schultheiss et al., 2004) and nPower and nAch (Wirth & Schultheiss, 2006). Of the remaining endocrine-related responses, there were mixed findings regarding nPower, epinephrine, and norepinephrine (McClelland et al., 1985; 1987) and one positive association between nPower and sAA (Wiemers et al., 2015). Collectively these findings do not allow for firm conclusions regarding these endocrine-related responses.

Overall, many of the studies underpinned by needs theory were not able to confirm study hypotheses through parsimonious and coherent statistical analyses that directly matched study

hypotheses. Instead, these studies heavily relied on post-hoc analysis of single timepoints, separate analysis of sub-samples, removal of participants, and flexible data analytic strategies. In contrast, other theory-based work, such as the AGT, SDT, and implicit theory studies employed much simpler conventional analysis that clearly matched study hypotheses. Thus, the evidence pertaining to needs theory-based motivation is not as robust as other theories. This point notwithstanding, it should be noted that explicit motivation (note that incremental and entity implicit beliefs are typically measured explicitly, despite their label) may have a stronger relationship with endocrine-related responses than implicit motivation. Implicit and explicit motivational systems largely operate independently and influence behaviour in different ways (Schultheiss, Patalakh, Rawolle, Liening & MacInnes, 2011), and this distinction may extend to physiological processes (Schultheiss et al., 2012). There may also be a lack of methodological sensitivity when measuring implicit motivation, compared to explicit motivation, thus leading to weaker associations with physiological responses. This presents an alternative explanation to the less conclusive evidence between needs theory and endocrine-related responses.

Strength and Quality of Evidence

Risk of bias was relatively low in the studies included in the review, with a few exceptions. The studies that scored lower on the bias inventory tended to be older (e.g., Dabbs et al., 1990; McClelland et al., 1980; McClelland et al., 1987) and is perhaps indicative of the evolving standards of reporting in contemporary research. There was evidence of risk of bias in blinding of experimenters to condition, statistical power, and reporting exact probability values. Regarding double-blinding, researchers should always consider the feasibility of double-blind designs to attain the highest quality research. At present, this methodological option is not commonplace in broader psychological research. In contrast, exact probability values and power analysis are increasingly a requirement of psychological research in general, particularly following the replication crisis in psychology. On a related note, the cortisol studies based on AGT, SDT and implicit needs theory all reported large effect sizes for their experimental studies (Hogue et al., 2017; Hogue et al., 2013; Reeve & Tseng, 2011; Yeager et al., 2016, study 1); however only two studies reported a power analysis, of which one was underpowered. It is not possible to draw any firm conclusions about the possibility of publication bias towards positive results, but it remains a threat to the validity and reliability of the observed effect (Button et al., 2013).

Concerns have been raised about the methodological validity, inferences that are made, and cases of circular reasoning in motivational research examining physiological markers (Richter & Slade, 2017). The motivation-based experimental conditions reviewed here demonstrated content and face validity, with studies frequently employing successful manipulation checks to demonstrate concurrent validity. Predictive validity was evident, for example, higher quality motivation predicting an attenuated cortisol response when faced with a moderate stressor, and greater nPower predicting higher testosterone and lower sIgA in more specific contexts. Finally, convergent validity was also demonstrated as the respective SDT, AGT and implicit theory constructs of autonomous functioning, task-involvement and incremental theory all produced a theoretically-coherent cortisol response to evaluative learning situations. Within the experimental studies, there was little evidence of circular reasoning, either within or between studies. All experimental designs followed a clear methodological pathway that contained a measure of motivation or a motivational manipulation, with the endocrine-related response of interest measured at baseline, and at least one further timepoint during the experimental procedure. There was no evidence of subsequent experimental attempts to use the endocrine-related response as a predictor of the experimental procedure, or as a motivational measure or manipulation. In conclusion, even though the experimental studies included within this review seem to free of the issues raised by Richter and Slade (2017), researchers should remain vigilant of their concerns.

Future Directions and Limitations

As discussed previously, none of the studies in this review included measures of behavioural outcomes or performance as part of their experiments (the win/loss studies had a pre-designated winner, so performance was not objectively comparable), despite several having an evaluative element in the experiment (e.g., juggling, problem solving). This systematic review suggests high quality motivation is implicated with an attenuated stress response. Investigating whether the physiological effects are associated with, or independent of, performance measures represents a worthwhile scientific endeavour. Relatedly, recent attempts have been made to integrate several conceptually-related motivation theories (Dweck, 2017; Vansteenkiste et al., 2014). Indeed, empirical studies are more frequently adopting integrated approaches to investigate motivation-related phenomena (e.g., Job, Sieber, Rothermund & Nikitin, 2018). Future research should consider investigating this multi-theoretical perspective to further

understand the relationship between theoretically distinct motivational constructs. Exploring whether there is an additive effect when integrating high-quality motivational constructs and measuring physiological outcomes, or whether there is a ceiling to downstream effects is one possible line of investigation.

There is evidence to suggest nAff is positively associated with an enhanced immune response. However, why the desire for affiliation and related constructs, such as feelings of relatedness and social support have such an effect of health and well-being remains unresolved (Taylor, 2007). Psychosocial states may trigger protective biological processes because of the enhanced risk of virus in social groups, relative to more isolated states (Cole et al., 2007). The motivational processes implicated in these relationships warrant further investigation. For example, is the relationship between nAff and sIgA mediated by fulfilment of the need, or is there a direct effect of nAff on sIgA independent of social contentment? In other words, does the desire for affiliation trigger a proactive biological response in anticipation of fulfilling that desire (direct effect), or is the biological response a reaction to satisfying the need for affiliation (indirect effect). This line of research has important implications for motivational science, and for broader social support theories and their associations with physical and mental health.

Finally, the present work was limited to published studies, dissertations and theses only. In addition, non-English language studies were not considered. Although this is an acceptable method for systematic review, it may represent an incomplete picture of the literature. Despite adherence to methodological guidelines, subjectivity always remains a threat to the validity of a systematic review (Eysenck, 1994). This threat was mitigated by using two reviewers to screen the studies down to the inclusion stage, and the third author also extracted a random sample of the included studies to check adherence to extraction protocols. It should also be acknowledged that the boundaries by which the inclusion criteria of both motivational constructs and endocrine-related responses were defined may be subject to interpretation. Nonetheless, although researchers may differ in their methodological approach to systematic review, it is unlikely the results will be affected by significant divergence (Nieminen, Nicklin, McClure & Chakrabarti, 2011).

Conclusion

This review has been compiled to provide a scientific assessment of the current state of the literature concerning multidimensional motivation and salivary endocrine-related responses.

There is experimental evidence that motivational constructs emphasizing higher quality motivation produce an adaptive cortisol response in evaluative tasks. The robustness of this conclusion is enhanced by either replication or evidence in similar contexts. There is also evidence that nPower is associated with lower, and nAff is associated with higher levels of sIgA. Evidence also exists within contextual situations, such as individuals high in nPower displaying increased testosterone when winning a contest, however the evidence was not conclusive. Overall, the growing body of work suggests distinguishing motivation of a higher and lower quality helps us understand physiological responses to psychological phenomena. This conclusion has important implications for improved human functioning, health and well-being.

Footnotes

[1]The decision to focus on salivary markers did not substantially change the results of the review. Consideration of non-salivary measures would have led to the inclusion of one cross-sectional study, which evidenced no significant associations between the need for affiliation and cortisol, epinephrine and norepinephrine in plasma (McClelland et al., 1993).

[2]As a predominant theory of motivation that is employed in a wide-range of theoretical and applied research, self-efficacy (Bandura, 1977) was included in the initial database search items. However, after reviewing the theoretical and methodological application of self-efficacy research at the full-text stage, the reviewers unanimously agreed that it did not qualify as a multidimensional motivational theory, and all studies relating to self-efficacy were excluded.

Chapter Three

Study Two

The Effect of Self-Determined Motivation on Self-Control Performance and Cortisol

Abstract

Self-control strength has long been conceptualized as dependent on a finite resource that once depleted can lead to performance decrements or maladaptive outcomes. Recently this theory has come under increased scrutiny, with authors questioning its parsimony and its existence. Utilizing a framework grounded in self-determination theory, this study examined whether a) manipulating participants' motivation would affect acute stress levels, measured by the stress hormone cortisol; b) Whether the motivational effect would influence self-control performance; and c) if this effect would persist over several self-control tasks. A single-blind randomized experimental design incorporating two motivational conditions (autonomy-supportive and controlled regulation) tested this hypothesis. Participants (N = 39) performed three sequential self-control strength challenges; a modified Stroop task followed by two wall sits. Salivary cortisol was measured at baseline, and after each of the wall sits. Participants' cortisol levels and self-control performance were both affected by the experimental manipulation. A repeated measures ANCOVA revealed a significant quadratic interaction (F (1, 32) = 5.40, p =.027, η_p^2 = .14). Participants in the controlled regulation condition experienced an increased, with autonomy supportive experiencing a decreased, cortisol response at timepoint 1. A mixed ANCOVA also revealed a significant effect for experimental condition on self-control performance (F (1,36) = 4.40, p = .043, η_p^2 = .11). Participants in the controlled regulation condition recorded greater wall sit performance in the first and second wall sits compared with the autonomy-supportive condition. Results support previous findings reflecting adaptive motivational effects on cortisol responses. However, performance on the self-control tasks was contrary to expectations with participants in the controlled condition outperforming those in the autonomy-supportive condition. In summary, increased self-control performance coincided with increased cortisol, suggesting an increased psychological and/or physiological cost for increased performance.

Keywords: self-determination theory, stress, ego depletion, organismic integration theory

Introduction

There are a number of epistemologically similar models of motivation that are unequivocal in suggesting that the quality of motivation (i.e., the reason underpinning a behaviour) is more important than the quantity of motivation when considering goal-directed behaviour and well-being. One of the leading theories that seeks to explain the quality of motivational regulation is self-determination theory, particularly a sub-theory: organismic integration theory (OIT; Ryan & Deci, 2017). The theory postulates that humans are growth-oriented and will, under appropriate conditions, naturally internalize socially sanctioned behaviour to become personally endorsed, valued and self-regulated (Ryan, Connell & Deci, 1985). The degree to which this process is completed or forestalled is a key component of organismic integration theory. Behaviour that is successfully internalized is regulated autonomously, with a sense of volition and choice. In contrast, partially internalized behaviour or behaviour that remains externally regulated is controlling in nature (Ryan & Deci, 2017). There is a wide-body of literature that supports the beneficial outcomes of autonomous motivational regulation. Although controlled motivation can facilitate superficial behaviour and performance (Cerasoli, Nicklin, & Ford, 2014), and energize short-term behaviour, it is often accompanied by psychological costs or ill-being (Ng et al., 2012). Only recently, however, have researchers begun to examine the underlying physiological impact of autonomous and controlled motivation, despite the opportunity for greater understanding of the psychological correlates of physical health. This study will therefore examine the effect of autonomous and controlled motivation on salivary cortisol secretion, a biomarker of acute stress, during tasks requiring self-control.

OIT subdivides controlled motivational regulation into two categories. External regulation is the most controlled form of regulation (hence, the least internalized form of regulation) and refers to engagement in an activity that is contingent on external rewards or to avoid punishment (Ryan & Connell, 1989). An example is a person who undertakes a mundane or unsatisfying task to receive payment or avoid punitive measures. Introjected regulation, on the other hand, refers to behaviour that has been partially internalized, yet is still controlling. It is distinct from external regulation in that the pressurizing contingencies underpinning behaviour are administered by the individual upon themselves, rather than from external sources (e.g., the avoidance of guilt or shame or to obtain self-worth; Deci & Ryan, 2000). For instance, introjected regulation might be a junior tennis player who plays through feelings of guilt as she

does not want to disappoint her parents. Autonomous extrinsic regulation is also divided into two motivational regulations. Identified regulation encompasses behaviour that is personally endorsed by the individual as being valuable or important (Ryan & Deci, 2017). An example of identified regulation would be consuming nutritious food choices because it is important to eat healthily. Finally, integrated regulation describes behaviour that is fully-endorsed, and integrated with all aspects of person's wider identity (Deci et al., 1994). If healthy behaviour became a large part of an individual's values, this would typify integrated regulation.

A wealth of empirical research has demonstrated the quality of motivation (how autonomously the activity is pursued) is more important that the quantity of motivation (higher degrees of motivation) in producing adaptive outcomes. A recent meta-analysis supported this assumption; higher quality autonomous motivation was associated with improved outcomes in several performance-related contexts (Cerasoli et al., 2014). For example, within organizational environments, increased autonomous motivation was associated with increased performance, particularly on tasks requiring creativity and cognitive flexibility (Gagné & Deci, 2005). Moreover, autonomous extrinsic regulation (i.e., identified and integrated regulation) may be most effective in improving performance on uninteresting tasks requiring disciplined task engagement. This proposition is supported by lab-based investigations demonstrating that the inclusion of meaningful rationales for completing an uninteresting computer task can promote internalization and autonomous regulation (Deci et al., 1994).

Demonstrating that increasing autonomous motivation leads to less demand on cognitive resources and persistence on uninteresting tasks has led researchers to consider the effect of autonomous motivation on self-regulatory processes. Self-control, colloquially known as willpower, is the capacity for an individual to alter their behavioural impulses to bring them into line with personally endorsed values, morals, ideals or social expectations, particularly in pursuit of long-term goals (Baumeister et al., 2007). Critically, self-control typically declines over repeated use (Hagger et al., 2010). In a classic series of studies, an experimental group performs two sequential, effortful tasks that required self-control. Meanwhile, a control group performs an identical second task; however, the first task, although conceptually similar to the experimental group's task, does not require self-control (viz., the sequential-task paradigm; Baumeister & Vohs, 2016a). When compared with the control group, the experimental group's performance on the second task is diminished due to additional exertion of self-control resources during the first

task. This performance decline is dubbed ego depletion. A meta-analysis of over 100 studies employing the sequential-task paradigm concluded that ego depletion was a robust, observable effect (d = 0.62; Hagger et al., 2010).

However, in recent years the ego depletion effect has become a hotly debated topic. A recent meta-analysis cast doubt on the size of the ego depletion effect (Carter, et al., 2015). Furthermore, alternative explanations have been presented that question the mechanisms behind ego depletion. Theorists have proposed that self-control may be more a question of resource allocation than limitation (Beedie & Lane, 2012), or that the decision to exert self-control is a function of opportunity costs (Kurzban et al., 2013). It has also been proposed that motivational and attentional processes are key mechanisms explaining the ego depletion effect (Milyavskaya & Inzlicht, 2016). The motivational proposition has been investigated using self-determination theory constructs as potential moderators for self-control performance. Several contextual inputs associated with supporting autonomous motivation have been used to influence ego depletion. For example, ego depletion can be reduced when participants are given appropriate rationales for the importance of the task, led to believe that performance would improve the longer they practiced (Muraven & Slessareva, 2003), provided with free choice (Moller et al., 2006) or receive instructions for the task that supported autonomy (Muraven et al., 2008). Holding autonomous reasons for undertaking an initial self-control task also leads to lower subsequent ego depletion (Muraven, 2008). However, the motivational benefit can disappear when participants are exposed to several self-control tasks (Graham et al., 2014; Vohs et al., 2012).

It remains poorly understood, however, why motivational and attentional processes influence the ego depletion effect. Early conceptualizations suggested self-control is sensitive to increases in stress, negatively affecting the ability to exert self-control (Muraven & Baumeister, 2000). For example, during the end-of-term exam period, university students reported high perceived stress, emotional distress and impaired performance on the sequential-task paradigm (Oaten & Cheng, 2005). Greater accumulation of chronic stress and negative life events are also associated with poorer trait self-control (Duckworth et al., 2013; Hamilton et al., 2014). Heart rate variability (HRV; a potential marker of stress) increases when self-control is activated and may therefore indicate a bi-directional relationship (Geisler & Kubiak, 2009; Reynard, Gevirtz, Berlow, Brown & Boutelle, 2011; Segerstrom & Solberg Nes, 2007).

If self-control and ego depletion are sensitive to stress, then the motivational regulation for engaging in the activity should influence this process. Indeed, work in this area has begun to explore the influence of motivation on physiological processes. A recent review explored the associations between neuroscience and self-determination theory (Di Domenico & Ryan, 2017). In addition to linking self-determined motivational constructs to neurological processes, the review also highlighted the key benefits of extending motivational research to biological processes. First, motivation and behavioural processes are mediated by biological and physiological functioning, thus investigating these processes provides a more complete account of how motivation promotes optimal human functioning. Second, examining internal physiological processes allows exploration beyond that which is achievable by self-report or behavioural measures. Third, examining physiological responses allows a fine-grained approach for examining the mechanisms that underpins human motivation. Furthermore, examination of physiological markers is an important step for the validation of motivational science. Drawing on the key benefits of biological investigation highlighted by Di Domenico and Ryan, the present investigation examined a key system implicated with optimal human functioning, namely the endocrine system, and specifically the stress hormone cortisol.

Cortisol is a key hormone activated during the stress response. In response to a stressful situation, the hypothalamic pituitary adrenal axis stimulates the release of cortisol via the adrenal cortex. Cortisol subsequently stimulates lipolysis, gluconeogenesis and catabolism of proteins into amino acids. In turn, this helps to repair damaged tissue and produce adenosine triphosphate, the body's key currency used in energy transfer. However, although cortisol is adaptive in helping the body to deal with short-term stress, prolonged exposure to stress can lead to psychological dysregulation (e.g., Burke, Davis, Otte & Mohr, 2005), and impair other physiological process such as immune functioning (Dhabhar, 2014). Thus, examination of cortisol represents a valid and reliable method of investigating how motivational processes are implicated in downstream physiological processes implicated with adaptive human functioning and improved health. Indeed, work has already demonstrated the potential importance of self-determined motivation and cortisol responses. For example, a learning environment that supported autonomous motivation was associated with enhanced behavioural and emotional engagement during a puzzle-solving task, relative to an environment facilitating controlled motivation. Moreover, autonomous conditions limited participants' cortisol response, whereas

controlled conditions augmented the cortisol response, relative to neutral conditions (Reeve & Tseng, 2011).

To summarise, autonomous motivation is associated with attenuating the cortisol response in social-evaluative environments, and conversely, controlled regulation is associated with provoking a cortisol response. Several studies have also demonstrated a consistent link between higher quality motivation and improved self-control performance (e.g., Moller et al., 2006). Furthermore, self-control performance has been shown to deteriorate under conditions of increased stress (Oaten & Cheng, 2005). Examining associations between higher quality motivation, self-control performance and the cortisol response may elucidate the proposition that stress is implicated in reducing self-control. Furthermore, research has demonstrated associations between self-determined motivation, stress and self-control; however, a concurrent examination is absent from the literature. Addressing this gap will form the central premise of the present study.

Research Hypotheses

The goal of this study was to examine how autonomous and controlled motivation concurrently affect the stress hormone cortisol and self-control performance. The primary hypothesis examined the association between motivational quality and cortisol. Based on previous research (Reeve & Tseng, 2011), it was predicted that participants who are experimentally manipulated into a condition emphasizing controlled regulation will experience an increased cortisol response, and conversely participants in the autonomy-supportive condition will experience an attenuated cortisol response.

Using self-control on an initial task typically reduces performance on a second self-control task (i.e., ego depletion; Baumeister & Vohs, 2016a; Hagger et al., 2010). However, ego depletion can be moderated by the underlying motivation for doing the tasks (Moller et al., 2006). It was, therefore, predicted that there will be no difference in initial performance in a first self-control task under autonomous and controlled motivation conditions. However, during a second self-control task, participants in the autonomy-supportive condition will perform better than those in the controlled regulation condition. In other words, participants in the autonomy-supportive condition will experience less ego depletion than participants in the controlled regulation condition (hypothesis 2).

Finally, research has suggested that, over time, motivational effects on ego depletion will dissipate. Specifically, although autonomous motivation can attenuate ego depletion in the short term, this increase in performance consumes greater self-control resources. As a result, fewer self-control resources are available for further tasks requiring self-control, and performance is impaired (Graham et al., 2014; Vohs et al., 2012). It is therefore predicted that although the autonomy-supportive condition will offer a short-term performance benefit on the sequential-task paradigm, that this effect will disappear on a third self-control task (hypothesis 3).

Method

Participants

Based on the effect size observed in similar studies manipulating motivation as the independent variable and measuring cortisol as the repeated measures outcome (e.g., Hogue et al., 2013, 2017; Reeve & Tseng, 2011), it was determined that 40 participants would be sufficient (α=.05, β=.80, *d*=.80; G*power, Heinrich-Heine-Universität). In total, data were collected from 41 participants (Male = 13; Female = 28). Two male participants were removed from the analysis; the first due to failed coercion into the controlled condition, and the second due to lack of engagement on the modified Stroop task resulting in significantly more errors (125) compared with the mean (*M* = 31, *SD* = 22). Analyses were subsequently performed on 39 participants aged between 18 and 51 years old (M_{age} = 22, *SD* = 5). Participants were invited to take part in a study measuring "the effect of cognitive and physical performance on hormones", and qualifying undergraduate students were eligible to receive course credit for participating.

Measures

Cortisol. Following the protocols outlined by the immunoassay kit manufacturer (Salimetrics, State College, PA, USA), saliva was collected via the passive drool method. Participants were seated, asked to void their mouth and allow saliva to pool in the well of the mouth without stimulation via facial movement. With their head tilted slightly forward, they were instructed to drool into the a 15ml cryovial approximately every 60 seconds. A target of 5ml of saliva collected over approximately three minutes guided collection, although as the saliva was collected unstimulated, it took some participants slightly longer to provide an adequate sample. Samples were taken at three timepoints, hereafter referred to as baseline, timepoint 1 (+19 mins) and timepoint 2 (+39 mins). Upon completion of the experiment, samples were immediately stored at -80^{o}c until assay.

Analysis of the cortisol samples was undertaken by the lead author using a commercially available salivary cortisol enzyme immunoassay kit (Salimetrics, State College, PA, USA). On the day of assay, the samples were thawed at room temperature (~22^{o}c) for a minimum of 1.5 hours. Upon thawing, 1000µl of saliva was centrifuged at 10,000 rpm for two minutes. The samples were analysed in duplicate in accordance with the manufacturer's instructions. The immunoassay measured cortisol in 25µL wells, with a sensitivity of <0.007µg/dL. Intra-assay and inter-assay precision coefficient of variation were 4.6% and 6% respectively. Spike recovery across eight samples averaged 104.9%, dilution recovery averaged 105.3% (four samples) and linearity of assay averaged 101.3% (nine samples).

Self-control. Self-control measures employed within the sequential-task paradigm are typically unrelated, hence different tasks were used (Chatzisarantis & Hagger, 2015). The first self-control task was a modified Stroop task (Wallace & Baumeister, 2002) written using Superlab (v4.5; Cedrus Corporation, San Pedro, CA), with the answers input using the Cedrus RB-530 response pad. The modified Stroop task was selected as it has been employed in numerous self-control studies (Hagger et al., 2010) and elicits a physiological stress response (Renaud & Blondin, 1997). Nine blocks of forty words coloured either red, green, blue, and yellow were displayed on a computer screen for a total of 800ms with a 500ms pause between words, upon which a control "+" was displayed in the centre of the screen in black ink. Responses were input by pressing the corresponding colour on the response pad.

In the modified version of the Stroop, participants were required to respond to the colour the word was written in and override the impulse to respond to the written name of the colour (i.e., for the word "GREEN" displayed in the colour yellow, the correct answer would be "yellow"). The exception to this rule was when the ink colour was red; participants were then required to input the semantic meaning of the word, not the colour of the text (i.e., the word "GREEN" in red ink, the correct response would be green). Self-control is required to override the general rule to identify the colour of the text, and instead identify the semantic meaning of words written in red ink. Performance was measured by comparing several dependent variables, including correct answers, number of errors and response times.

The second and third self-control tasks were a wall-sit postural endurance task, which requires self-control exertion to override a desire to stop and recover from the discomfort (Boat & Taylor, 2017). The procedure required the participant to place their back and shoulders against

a wall within the laboratory and assume a sitting position with their knees bent at 90 degrees and their thighs parallel to the floor. Once they had assumed the position, the experimenter started a stopwatch and they were required to hold this position for as long as they were able. Failure was deemed to occur when the participant's form deviated from the original position, and they were unable to correct within two seconds when prompted by the experimenter. Once failure had occurred, the total time elapsed was recorded to the nearest second. The first wall sit was deemed the dependent measure of the ego depletion effect (hypothesis 1), and the second wall sit determined if motivational effects on self-control performance persisted over time (hypothesis 2).

Manipulation checks and covariates. ***Intrinsic motivation.*** The Intrinsic Motivation Inventory (IMI; McAuley, Duncan, & Tammen, 1989) was administered to assess if motivation had been successfully manipulated. The questionnaire consists of three subscales rated on a five-point likert-type scale ranging from 1 (*Not at all true*) to 5 (*Completely true*): interest/enjoyment (eight items: e.g., "This activity was fun to do"), value/usefulness (nine items: e.g., "I think this is an important activity"), and perceived choice (eight items: e.g., "I felt that I had to do this activity"). The interest/enjoyment subscale is considered the measure of intrinsic motivation (Ryan, 1982), perceived choice considered to be predictive of self-report and behavioural measures of intrinsic motivation, and value/usefulness representing the internalization of an uninteresting task (Deci et al., 1994). The scale has shown good reliability in similar studies measuring intrinsic motivation towards computer tasks (e.g., Moller et al., 2004; $\alpha = .88$; see Appendix Three).

Mood. The Brief Mood Introspection Scale (BMIS; Mayer & Gaschke, 1988) assessed mood to ensure any experimental effects were not attributable to affecting mood. Participants rated 16 items (e.g., "lively", "drowsy") describing how well each item described their present mood, using a 4-point likert scale ranging from 1 (*definitely do not feel*) to 4 (*definitely feel*). Items describing negative affect are reverse scored when calculating the total score. The scale measures the sub-scales of valence and arousal and has good reliability ($\alpha = .76$ & $\alpha = .83$ respectively, Muraven & Slessareva, 2003; see Appendix Four).

***Perceived mental and physical exertion*.** The Borg single-item CR-10 scale (Borg, 1998) was used to measure perceived mental and physical exertion on the Stroop tasks and wall sit, respectively (see Appendix Five & Six). The scale was administered to assess if there was a

difference in perceived exertion between conditions, and between the congruent and incongruent Stroop. The scale uses a single 10-point scale, with higher scores indicating more perceived physical exertion (0 = *extremely weak*; 10 = *absolute maximum*).

***Trait autonomy*.** The Index of Autonomous Functioning (IAF; Weinstein, Przybylski & Ryan, 2012) was used to control for global levels of trait autonomy. The 15-item scale consists of three subscales; self-congruence (five items: e.g., "I strongly identify with the things that I do"); susceptibility to control (five items: e.g., "I do things in order to avoid feeling badly about myself"); and interest taking (five items: e.g., "I like to investigate my feelings"). Participants were asked to rate their general experiences towards each of the statements on a 5-point likert scale ranging from 1 *(Not at all true*) to 5 (*Completely true*). The scale has demonstrated good reliability across a range of studies (α = .81-83, Weinstein et al., 2012; see Appendix Seven).

Procedure

Data collection commenced at either or 11:00am or 12:00pm to control for the cortisol diurnal profile. As per cortisol enzyme immunoassay kit manufacturer recommendations (Salimetrics, State College, PA, USA), participants were instructed not to eat a major meal 60 minutes before sample collection, or to consume alcohol 12 hours prior. In addition, it was requested that participants abstain from any food or drink other than water for one hour; to not eat a major meal at least two hours; and to abstain from alcohol, nicotine or physical activity for 24 hours prior to the study. These aspects were checked prior to the along with a question asking for details of any prescription medication they were taking, and their awakening time that day experiment (see Appendix Eight). Participants then provided a baseline measure of saliva and completed the IAF.

Participants were then randomly assigned into one of two experimental conditions; autonomy-supportive and controlled regulation. In a procedure first utilized by Legault and Inzlicht (2012), participants were offered a choice of a forthcoming task. The options were (a) The Mental Distraction Game; (b) A Game of Accuracy; c) Ignore Your Impulses, and (d) Cognitive Response Latency Test (see Appendix Nine). Unbeknown to the participants, these were all different names for the modified Stroop task they would all perform. In both conditions, participants were presented with the four options printed clearly on a sheet of A4 paper. Participants in the autonomy-supportive condition were offered a free choice between the

options. It was emphasized they would receive feedback to indicate how successful they were at the relevant skill to enhance the meaningfulness of the task.

To manipulate participants into the controlled regulation condition, participants were presented with the same options offered in the autonomy-supportive condition. However, upon presentation of the four options, the experimenter verbally explained that most participants had chosen options "a", "b" or "c", and that it would help the researcher to balance the experimental conditions if they chose option "d", the Cognitive Response Latency Test, which was ostensibly the least desirable task (nonetheless, two participants in the autonomy-supportive condition freely chose this option). However, it was stressed to the participant that the final decision was theirs, and participants then checked the option they wanted to perform. This was done to induce a feeling of guilt and coercion when selecting the task (i.e., controlling regulation). Aside from the experimental manipulation, all other aspects of the autonomy-supportive and controlled conditions were identical.

The participants were then presented with the instructions for the modified Stroop task (see Appendix Ten). Before undertaking the modified Stroop, participants were allowed a trial consisting of 40 words. The trial was identical to the main task, except it ran at the slower rate of 1500ms to input a response, with a 500ms pause between words. Participants were allowed as many trials as they wanted to ensure they understood the task and given the opportunity to ask questions to clarify any aspects. When they were ready to proceed, participants began the modified Stroop task, which ran for approximately 10 minutes. On completion of the modified Stroop, participants indicated the mental exertion the task required using Borg's single-item CR-10 scale (Borg, 1998). They were then asked to complete the first wall sit, and upon completion they indicated how much physical exertion the wall sit had required via Borg's single-item CR-10 scale (Borg, 1998). Following this, they provided a second saliva sample.

Participants then performed a congruent Stroop as an active control task. This version of the Stroop followed the same timing intervals as the modified Stroop; the only deviation being colours and words were congruent throughout the task (i.e., the word BLUE in blue ink). Studies have shown the congruent Stroop does not require self-control exertion, and when presented after the modified Stroop is not significantly stressful (Renaud & Blondin, 1997). The congruent Stroop was administered as an active control task to standardize the activity of the participants between the timepoint 1 and timepoint 2 saliva samples, so the timing between these timepoints

was similar to baseline and timepoint 1. They were given a task to complete so they would not engage in other activities that might affect their stress levels (e.g., check their mobile phone). Upon completion, participants indicated how much mental exertion the task required. Participants were then asked to perform a second wall sit that was identical to the procedure administered for the first, and upon completion indicated how much physical exertion the task required. For the final part of the experiment, participants completed the IMI and BMIS. Once the questionnaires were completed, the participants provided a final saliva sample saliva, were debriefed as to the true nature of the experiment and thanked for their participation.

Data Analysis

The hypotheses were explored using a repeated-measures ANOVA, thus controlling for individual differences in the dependent measures of cortisol and self-control performance. Prior to conducting the main analysis, potential covariates were checked. For cortisol, several covariates were checked that have the potential to affect data, that did not preclude participation in the study. These included abstinence from smoking or brushing teeth for 30 minutes; no food or drink other than water for one hour; to not eat a major meal at least two hours; any nicotine or alcohol consumed in the previous 12 hours; any physical activity undertaken in the previous 12 hours; a list of any medication and the participants waking time. The participants waking time was also subtracted from the time the baseline saliva sample was taken to control for diurnal variation in cortisol. The potential for covariates to affect the cortisol response was checked by regressing the cortisol taken at timepoint 1 on baseline cortisol and the covariates. Any significant ($p < .05$) covariates that emerged from this analysis were subsequently included in the repeated-measures ANOVA.

Potential covariates of wall sit performance, specifically gender, age or physical activity undertaken in the previous 12 hours were explored as predictors of the performance on the first wall sit, with significant ($p < .05$) covariates subsequently used in the main repeated-measures ANOVA analysis. Finally, the dependant measures of modified and congruent Stroop performance, including number of correct, incorrect and no responses, and reaction times were individually checked using one-way ANOVAs.

Results

Preliminary Analysis

Means, standard deviations, bivariate correlations and Cronbach's alpha coefficients for all variables are presented in Table 3.1. All data were normally distributed and had homogenous variances, aside from a positively skewed distribution of the cortisol samples. Following established guidelines (Smyth et al., 2013) four participants were removed due to the participant either awakening less than 60 minutes before the experiment (1 case), or their baseline cortisol being greater than three standard deviations above the mean (3 cases). Despite the removal of these outliers Shapiro-Wilk tests indicated that cortisol at baseline (W (17) = .82, p = .004), timepoint 1 (W (18) = .76, p < .001) and timepoint 2 (W (18) = .87, p = .017) were not normally distributed. Therefore, logarithmic transformation of the data was administered (Log-10), which normalized Shapiro-Wilkes distribution at baseline (W (18) = 0.93, p = .183), timepoint 1 (W (17) = .95, p = .504) and timepoint 2 (W (17) = .95, p = .513). Further data checks revealed Levine's test of homogeneity of variance was maintained (F (1,33) = 0.03, p = .862), and there was equal covariance across groups (Box's M = 6.24, p = .467).

Data Checks

The descriptive statistics for the data checks between conditions are shown in Table 3.1. There were no significant differences between conditions in dispositional autonomy, mood or perceived effort, suggesting that the experiment did not unwittingly manipulate mood or effort, and there were no pre-existing differences in dispositional autonomy. There were non-significant differences between the three dimensions of intrinsic motivation, suggesting the experimental manipulation had not led to differences in autonomous motivation towards the modified Stroop. Finally, as expected, there was a significant difference in perceived mental exertion measured using the Borg-CR10 scale between the congruent and modified Stroop (F (1,37) = 79.18, p < .001, η_p^2 = .68).

Cortisol

First, potential covariates were examined that might affect cortisol responses. This list consisted of abstinence from smoking or brushing teeth for 30 minutes; no food or drink other than water for one hour; to not eat a major meal at least two hours; any nicotine or alcohol consumed in the previous 12 hours; any physical activity undertaken in the previous 12 hours; a list of any medication and the participants waking time. Furthermore, participants waking time was subtracted from the time they provided their baseline saliva sample, to control for diurnal variation. A multiple linear regression was then conducted, regressing cortisol at timepoint 1 on

Table 3.1

Descriptive Statistics for the Study Variables Between Autonomy Support and Controlled Regulation Conditions

	Autonomous	Controlled											
	M(SD)	*M(SD)*	*p=*	1	2	3	4	5	6	7	8	9	10	α
1, SC (IAF)	3.80(.51)	4.02(.34)	.12	-										.65
2, StC (IAF)	2.64(.69)	2.72(.73)	.73	.11	-									.64
3, IT (IAF)	3.45(.79)	3.81(.86)	.19	.44**	.49**	-								.83
4, I/E (IMI)	2.61(.65)	2.50(.86)	.65	-.23	.15	-.003	-							.91
5, V/U (IMI)	2.93(.58)	2.92(.75)	.95	-.20	.33*	.06	.44**	-						.82
6, PC(IMI)	4.45(.49)	4.53(.49)	.66	-.08	-.17	-.25	.09	.000	-					.75
7, BMIS	3.05(.45)	2.99(.37)	.68	-.04	-.09	-.05	.37*	-.02	.22	-				.85
8, Mental Borg 1	5.68(1.57)	5.58(2.12)	.86	.02	-.03	.11	.07	.16	-.31	-.23				-
9, Physical Borg 1	5.42(2.89)	6.10(1.92)	.39	-.14	-.02	.09	.23	-.02	.03	.05	.40*			-
10 Mental Borg 2	2.50(1.30)	3.08(2.15)	.32	.12	.06	.18	.01	.18	-.46**	-.30	.40*	.18		-
11, Physical Borg 2	5.97(2.86)	6.75(1.59)	.30	-.14	-.20	.07	.20	-.16	-.06	.05	.39*	.85**	.31	-

*$p < .05$; **$p < .01$; SC = self-congruence; IAF = index of autonomous functioning; StC = susceptibility to control; IT = interest taking; I/E = interest/enjoyment; IMI = intrinsic motivation inventory; V/U = value/usefulness; PC = perceived choice; BMIS = brief mood introspection scale;

the potential covariates and cortisol at baseline to see if any of the potential covariates affected change in cortisol response. None of the covaries were significant, except for waking-to-baseline saliva time. Therefore, this was included this as a covariate in the main model.

Due to violation of sphericity ($\chi^2(2) = .64$, $p = .001$), the Huynh-Feldt correction was reported for the main effect. A repeated measures ANCOVA, with experimental condition as the independent variable, cortisol as the repeated measures factor, and waking to baseline saliva time as the covariate using the Huynh-Feldt correction showed a non-significant within person effect (F (1.62, 51.91) = 1.70, p = .197, η_p^2= .05). Furthermore, there was a non-significant between person effect (F (1, 32) = 0.17, p = .679, η_p^2 = .005). However, there was a significant quadratic interaction for the experimental condition on participant's cortisol levels, controlling for waking-to-baseline saliva time (F (1, 32) = 5.40, p =.027, η_p^2 = .14), with a graphical representation shown in Figure 3.1. Including the outliers discarded during the data check did not meaningfully affect the quadratic effect (F (1, 34) = 4.18, p = .049, η_p^2 = .11). The baseline cortisol value for the autonomous and controlled condition respectively were 5.52 and 5.70. Participants in the autonomy-supportive condition showed a decrease in cortisol (5.00, 95% CI = 3.15, 6.86) at timepoint 1, which increased to 6.34 (95% CI = 4.48, 8.20) at timepoint 2. In the controlled regulation condition cortisol increased to 6.51 (95% CI = 4.70, 8.31) at timepoint 1, with this increase remaining stable at timepoint 2 (6.49, 95% CI = 4.68, 8.30).

Self-Control Performance

Modified Stroop. A full list of congruent and modified Stroop dependent variables is listed in Table 3.2. As expected, there were non-significant differences in performance between conditions for the modified Stroop, which was administered as the first part of the sequential-task paradigm.

Wall sit. First, potential confounds of age, gender or physical activity undertaken in the previous 12 hours (PA<12) were checked to see if they affected wall sit performance. A multiple linear regression with age, gender and PA<12 as predictors of wall sit performance revealed a trend (F (3,35) = 2.17, p = .11), that was driven by PA<12 (t = 2.11, p = .04, β = .33), and this was therefore included as a covariate in further analysis. Data were analysed using a mixed-ANCOVA, with experimental manipulation (autonomous versus controlled) as the between-participants factor and wall sit performance as the repeated measures factor. The results revealed an effect for performance over time that met the level of a trend (F (3,36) =

Figure 3.1.

Change in the repeated measures of cortisol between conditions with standard error bars

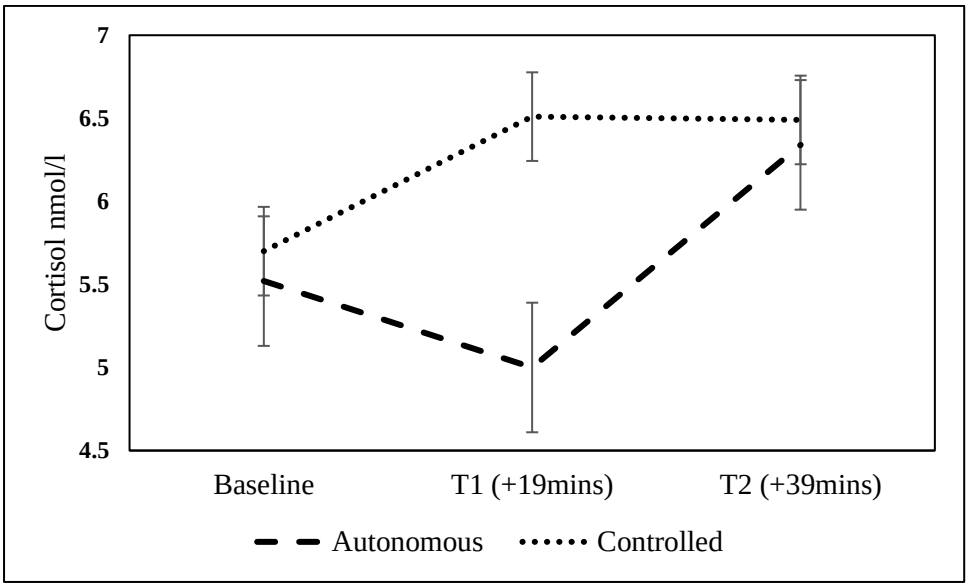


Table 3.2

Dependent Variables for the Stroop Task

	Autonomous		Controlled				
Modified Stroop	N *M (SD)*	RT *M (SD)*	N *M (SD)*	RT *M (SD)*	*F* =	*p* =	η_p^2 =
Correct Responses	285.00(30.29)	718.68(55.71)	282.25(33.19)	702.25(51.31)	0.07	.789	.002
Incorrect responses	29.58(20.09)	748.84(59.69)	31.80(24.13)	745.25(74.07)	0.10	.757	.003
No Response	45.42(22.46)	-	45.95(34.14)	-	0.00	.955	.001
Congruent Stroop*							
Correct Responses	342(9.68)	593.00(61.73)	341.20(10.73)	588.2(54.03)	0.06	.812	.002
Incorrect responses	14.56(9.18)	575.5(71.86)	13.80(8.76)	595.80(87.08)	0.07	.797	.002
No Response	3.44(4.00)	-	5.00(6.31)	-	0.80	.377	.022

Notes: N=number of responses; RT=reaction time in milliseconds; all p-values >.20. *One participant's congruent Stroop data in the autonomous condition was not available due to computer error.

2.91, p = .096, η_p^2 = .08), with participants performing better in the first wall sit (M = 141.1 seconds, 95% CI = 123.3, 158.9) than they did in the second wall sit (M = 111.4, 95% CI = 95.9, 126.8). A main effect of experimental condition was also significant (F (1,36) = 4.40, p = .043, η_p^2 = .11). Participants in the autonomous condition recorded wall sit times of 111 (95% CI = 88.9, 132.3) seconds, compared to 142 (95% CI = 120.7, 163.0) seconds in the controlled condition. Finally, as can be seen in Figure 3.2, the time by condition interaction was not significant (F (3,35) = 0.139, p = .711, η_p^2 = .01).

Post-Hoc Analysis

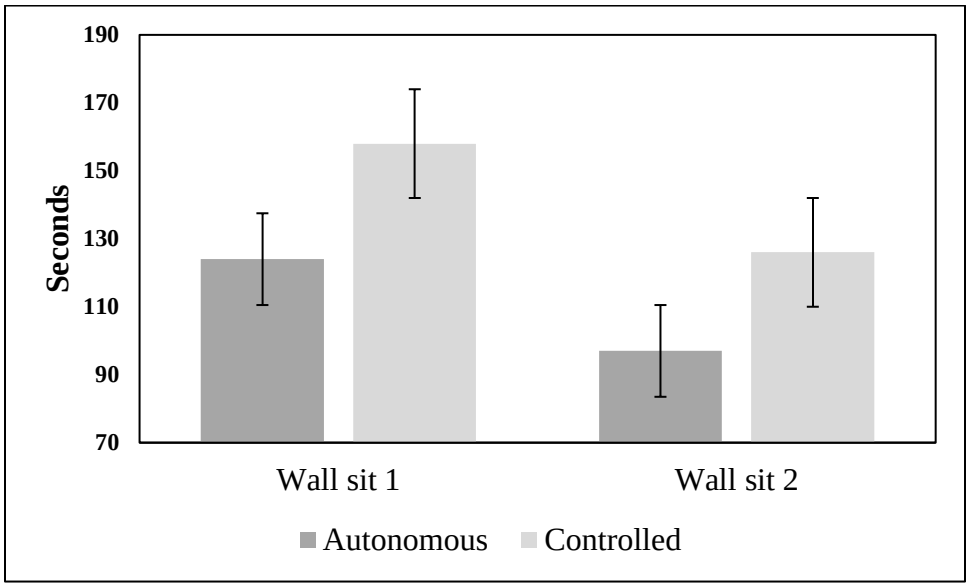
After reviewing the unexpected result of participants in the controlled condition performing better than autonomy-supportive, it was speculated that the increase in cortisol at timepoint 1 that coincided with completion of the first wall sit could have energized performance. Therefore, a linear regression was conducted, with timepoint 1 cortisol as the predictor, and the first wall sit as the outcome, which suggested cortisol positively predicted wall sit performance (F (1, 36) = 4.09, p = .051, β = .32). When using the Log-10 transformed scores, this reduced the significance to the level of a trend (F (1, 36) = 2.88, p = .099, β = .28).

Discussion

The present research examined autonomous and controlled motivation, and its subsequent effect on cortisol and self-control performance. The primary hypothesis examined how manipulating a participant's motivation affected their acute stress response, measured via cortisol. Supporting the primary hypothesis, there was a significant difference in cortisol between experimental conditions. Participants' cortisol levels increased in the controlled regulation condition and decreased in the autonomy-supportive condition. Further hypotheses compared the effect of autonomous and controlled regulation on self-control performance within the sequential-task paradigm. Although a significant difference in performance on the wall sit tasks was observed, the effect contradicted predictions - participants in the controlled condition achieved greater wall sit times than those in the autonomy-supportive condition. The final hypothesis examined self-control performance beyond the sequential-task paradigm. Again, the results contradicted predictions; the superior wall sit performance of the controlled participants observed between conditions persisted to the second wall sit. In the present study, when participants autonomy was supported by offering the perception of choice for engaging

Figure 3.2.

Wall sit performance between conditions with error bars representing the standard error



in a self-control task, this coincided with a reduced cortisol response. Conversely, when participants were coerced into choosing an ostensibly less favourable self-control task, they experienced an increase in cortisol. This supports a growing body of work demonstrating that higher quality motivation is adaptive in reducing the cortisol response in evaluative situations (Hogue et al., 2013, 2017; Reeve & Tseng, 2011; Yeager et al., 2016). Furthermore, these results validate the theoretical distinction that engaging in activities for more autonomous reasons has implications for improved health. Self-determination theory posits that behaviour underpinned by autonomy-supportive environments is positively associated with growth, integrity and well-being (Ryan & Deci, 2017). The release of cortisol is an adaptive response designed to mobilize resources to cope with a perceived threat. However, frequent activation of the HPA axis has implications for mental and physical health (McEwen, 1998). Therefore, the autonomy-supported attenuation of the stress response during socially evaluative tasks validates a key theoretical tenant that implicates autonomous motivation with improved well-being and physical health.

There are important distinctions that differentiate the current work with the extant literature. Most notably is the subtlety of the experimental manipulation. In the present study, a single "dose" of motivational manipulation was used, and aside from the manipulation, the experiment was identical between conditions. The experimental effect of cortisol observed in the study was significant; however, the effect size was not as large as effects observed in other studies manipulating the quality of motivation (Hogue et al., 2013; Hogue et al, 2017; Reeve & Tseng, 2011; Yeager et al., 2016). Although this may be perceived as a weakness, this smaller effect is arguably due to the subtlety of the experimental manipulation in the present study. This contrasts with the motivational environments used in other studies where the motivational salience was consistently reinforced throughout the task. The subtlety of the manipulation and its effect of cortisol levels can be viewed as a significant strength of the present work. It implies that by altering the framing of a task, it can not only influence performance, but also the physiological response. Moreover, it offers an explanation as to why cortisol levels at timepoint 2 returned to levels that were not statistically different between conditions or from baseline measures. As the motivational manipulation was not reinforced throughout the task, it is likely the motivational salience dissipated by the time the final measure of saliva was taken, 39 minutes after the experimental manipulation. This is important as cortisol levels are assumed to return to

pre-stressor levels around 40 mins after the end of the stressor (Dickerson & Kemeny, 2004). It is also important to note that this study was the first to test the effect of high quality motivation on cortisol response individually participants. Previous studies (Hogue et al., 2013; Hogue et al, 2017; Reeve & Tseng, 2011; Yeager et al., 2016) have all conducted their experimental manipulation concurrently in large groups of participants. This has the obvious benefit of collecting a large amount of data concurrently, but it also elicits the potential for a greater subjective feeling of social-evaluative threat. This is important as social-evaluative threat and uncontrollability, which each elicit an additive effect on the cortisol response (Dickerson & Kemeny, 2004). Conducting experiments in large groups most likely amplifies the social-evaluative threat due to the presence of a peer group. By testing participants in isolation, it suggests that high-quality motivation modifies the cortisol response in situations where social-evaluative threat is dampened. It's is important to note that in the present study, the experimenter was present throughout the procedure, and therefore social-evaluative threat would not be completely eliminated. Nonetheless, the results of the present study suggest that high-quality motivation may affect uncontrollability and social-evaluative threat differently, and future research should aim to tease out these differences.

Our second hypothesis stated that performance on the wall sit tasks would differ between experimental conditions. Although a difference between conditions was observed, the direction of the effect was contrary to predictions. In the controlled condition, participants performed better on the wall sit task than participants in the autonomy-supportive condition. Although this finding was unexpected, motivation undertaken for more controlled reasons can still motivate people to achieve positive outcomes, even when compared to more autonomous motivation (Ryan & Connell, 1989). In a recent meta-analysis, autonomous motivation was associated with higher quality performance (i.e., creativity, assembly quality, writing a research proposal). On the other hand, more controlled forms of motivation were associated with better performance on less complex tasks with a focus on outcomes where performance was measured using a discrete variable (Cerasoli et al., 2014). In the present context, the wall sit would better fit Cerasoli and colleague's meta-analytical data coded definition of less complex tasks. Supporting this proposition, pleasing the experimenter can act as a strong motivator, particularly on tasks requiring self-control (Inzlicht, Schmeichel & Macrae, 2014). Participants in the controlled condition were coerced into helping the experimenter, and therefore may have received a greater

quantity of motivation, albeit motivation that was lower quality. In conclusion, although findings of the present work contradict the extant literature, there are theoretical motivational and self-control related explanations for the unexpected effect.

Our third hypothesis concerned motivational persistence between groups. It has been argued that motivation can ameliorate the ego depletion effect in the short-term, however, existing research suggests that this effect will disappear over time (e.g., Graham et al., 2014). In the present study, the difference in wall sit performance remained consistent between groups, suggesting the motivational effect persisted between wall sits. If self-control is a limited resource, then participants who used more of this resource initially (i.e., the controlled motivation group) should experience reduced performance on subsequent tasks; however, this study rejected this assumption. The present results bucked the trend of motivational effects dissipating over repeated self-control tasks (Graham et al., 2014; Vohs et al., 2012), and therefore tacitly questions the idea of self-control as a limited resource.

However, there are potential caveats to be considered when interpreting this conclusion. First, it has been argued that exposure to sequential, identical self-control tasks can introduce confounds that may mask ego depletion effects (Chatzisarantis & Hagger, 2015). Confounds that may have been present in the current design include, but are not limited to, development of response strategies, enhanced levels of boredom, practice effects and depleted physical capacity. Although it was not presumed that these had any effect within the present work, replicating the present design with different self-control tasks would remove this potential limitation. Second, several studies have demonstrated that making a choice before tasks requiring self-control can lead to ego depletion compared to a low- or no-choice conditions (Baumeister et al., 1998; Bruyneel, Dewitte, Vohs & Warlop, 2006; Vohs, et al., 2008). In the present study, participants in the autonomy-supportive condition chose a cognitive task; conversely, this decision was removed in the controlled regulation condition. However, this counterargument is unlikely; decision-related depletion would most likely have been evident in the modified Stroop task performed directly after the decision (e.g., Baumeister et al.,1998, experiment 2). Furthermore, choice-related ego depletion typically requires multiple decisions (e.g., Vohs et al., 2008). Therefore, although choice cannot be ruled out as an explanation for the results, the supporting evidence is relatively weak.

It is also important to consider whether there was evidence of a relationship between cortisol and self-control performance. It was speculated that the increase in cortisol observed in the controlled condition may have energized self-control performance. Post-hoc analysis offered some support for this proposition - an increase in cortisol was associated with increased performance on the first wall sit. Previous research (e.g., Hogue et al., 2013; Reeve & Tseng, 2011) examining the quality of motivation and cortisol change has not examined the concurrent effects on performance. Therefore, there is little evidence examining the potential interaction between motivational processes, physiological responses and task performance. There is however evidence suggesting physiological processes are important for self-control performance. An evolutionary conceptualization of self-control offers the suggestion that resources may be allocated, rather than limited, and physiological processes are acknowledged as mechanism in this process (Beedie & Lane, 2012). Cortisol is responsible for mobilizing energy resources to respond to a potential threat (viz., general adaptation syndrome, Selye, 1946). Therefore, an evolutionary adaptive stress response may lead to allocation of resources to cope with task demands, and potentially energize self-control performance. Therefore, this may explain the unexpected findings of self-control performance in the present study.

Although it is tempting to suggest this increase in cortisol may have wider implications for self-control performance, caution is advised before generalizing this conclusion. Due to the physical characteristics of the wall sit, it would be premature to suggest stress-related effects extend to all domains of self-control performance. Moderate increases in stress levels can facilitate increased physical performance levels, particularly in disciplines that require strength and gross physical effort (Crewther, Heke & Keogh, 2011; Hanton, Mellalieu & Williams, 2015). However, the gross physical effort required by the wall sit contrasts with physical self-control tasks that recruit far fewer muscles (e.g., handgrip; Graham et al., 2014) and tasks that require fine motor skills (e.g., a dart throwing task; McEwan, Ginis & Bray, 2013) that may not be positively influenced by cortisol. Furthermore, cognitive tasks may not benefit from cortisol to the extent that gross physical tasks do. Stress has a complex relationship with cognitive performance across numerous tasks and environments (Cohen, 1980; McEwen, & Sapolsky, 1995), however there is strong evidence suggesting an inverted-U relationship between acute stress and cognitive performance (Lupien, Maheu, Tu, Fiocco & Schramek, 2007). Although this line of reasoning suggests an energizing effect for moderate amounts of cortisol, it is unclear if

cognitive tasks requiring self-control would replicate this relationship. Finally, an adaptive effect of cortisol on self-control performance contrasts with the assumed negative relationship between stress and self-control (Muraven & Baumeister, 2000), and indeed some of the empirical research (e.g., Oaten & Cheng, 2005). It should also be noted that Muraven and Baumeister's conceptualizations of a deleterious effect of stress on self-control performance does not make a clear distinction between acute and chronic stressors, or as in the case of Oaten and Cheng relates to acute naturalistic stressors. The present work used a lab-based stressor, and although this limits its generalizability, it is comparable to the majority of self-control research that has been undertaken in laboratory settings (Hagger et al., 2010). In conclusion, the energy mobilized by the cortisol response, combined with a physical task that was suited to recruiting these resources, may offer an alternative explanation for the results of the study. However, further research is required before this proposition can be generalized to further domains of self-control performance.

Limitations and Future Directions

There are several limitations of this study that should be identified. First, a control group was not used, which might have provided a clearer indication as to how motivation affected the cortisol response and self-control performance. Second, the study would also have benefitted from further details about the participant's physical activity levels. This would allow us to control for individual differences in physical activity on wall sit performance, thus controlling for physical fitness between conditions. Third, the experiment was conducted by the lead researcher who was not blinded to the aims of the study. While every effort was made to ensure consistency between conditions, this nonetheless increases the threat to internal validity. Fourth, the manipulation check administered to detect a difference between groups was not successful. This limits the scope of the conclusion; however, the failure may be a procedural weakness than a conceptual one. The IMI was administered at the end of the experiment, and therefore the motivational effects may have dissipated; a conclusion supported by the lack of a significant difference in cortisol at timepoint 2. Furthermore, participants may have been confused as to which aspect of the experiment the manipulation check related to. These points notwithstanding, it should not be assumed the success of a manipulation check automatically improves the quality of the study (Hauser, Ellsworth & Gonzalez, 2018).

One of the key questions raised by this study is whether the difference in cortisol levels influenced wall sit performance (i.e., a physiological effect), or whether the difference in wall sit performance was independent of the observed changes in cortisol (i.e., a psychological effect). Future work should test the causal processes between stress and self-control performance. Manipulating the salience of the stressor and measuring the subsequent impact on a variety of physical and cognitive self-control tasks would appear a fruitful avenue for future research.

Conclusion

The examination of motivation in relation to cortisol and concurrent effects on self-control tasks suggests several potential effects. The results of this study support a growing body of literature documenting the physiological benefits of higher quality motivation. Participants exposed to a condition emphasizing autonomy support experienced an attenuated cortisol response in contrast to those in the controlled regulation condition who experienced an increase in cortisol. However, contrary to predictions, participants in the controlled regulation condition benefitted from an increase in self-control performance when compared the autonomy-supportive condition. In summary, in the controlled regulation condition, increased self-control performance coincided with increased cortisol, suggesting participants may have paid an increased psycho-physiological cost for their increased performance.

Chapter Four

Study Three

The Motivational Effect on Self-Control Performance and Cortisol: A Replication and Extension

Abstract

The replication crisis in psychology has caused a great deal of consternation among scientists, with self-control in particular exposed to increased scrutiny in recent years due to meta-analysis and multi-lab replications casting doubt on the reliability of the effect. The present study replicated and extended the method used in Chapter Three, with the aim of replicating the cortisol effect observed, and further investigating the unexpected self-control results. Participants (N = 70) were randomly allocated to one of three conditions: Controlled regulation, autonomy-supportive or a control group. Performance was recorded over three self-control tasks - a modified Stroop, wall sit and a plank, with salivary cortisol measured concurrently. A repeated measures ANOVA revealed a significant linear within subject contrast (F (2, 57) = 4.45, p = .016, η_p^2 = .14), with a significant linear decline in cortisol observed in the control condition. Furthermore, support for the effect of autonomy support on self-control performance was observed. A one-way ANOVA revealed a performance difference between experimental conditions (F (67,2) = 2.88, p = .063, η_p^2 = .08), that on further investigation was driven by autonomy-supportive participants performing better than those exposed to controlled regulation (p = .055). As predicted, this performance difference did not persist to the second self-control task, the plank (F (64,4) = 0.41, p = .664, η_p^2 = .03). Although the cortisol results do not replicate the observations of Chapter Two and Three, the present results do support the idea that autonomous motivation is an important mechanism in self-control performance.

Introduction

The replication crisis in psychology (Simmons, Nelson & Simonsohn, 2011) has led to a greater awareness of what constitutes good science, with numerous essays and commentaries illustrating epistemological and pragmatic considerations for improving research practices in psychology (e.g., Finkel et al, 2015; Finkel, Eastwisk, Reis, 2017; LeBel, Berger, Campbell & Loving, 2017). Self-control, and specifically, the ego depletion effect, has come under increased scrutiny, with conceptual and philosophical issues highlighted (Iso-Ahola, 2017: Lurquin & Miyake, 2017). The debate surrounding the ego depletion effect can be traced back to a meta-analysis documenting the ego depletion effect across a variety of spheres (Hagger et al., 2010). Despite the seemingly robust finding, subsequent meta-analyses questioned the existence of the effect (Carter & McCullough, 2014; Carter et al., 2015). However, further meta-analytical work called into question the methods and conclusions arrived at by Carter and colleagues (Cunningham & Baumeister, 2016; Inzlicht, Gervais, & Berkman, 2016). The water was further muddied by the failure of a high-profile pre-registered multi-lab experiment to detect the ego depletion effect (Hagger et al., 2016).

The debate surrounding the replicability of the ego depletion effect led to it being dubbed "the poster child for the replication crisis" (Baumeister, Tice & Vohs, 2018, pp 143), and as of the time of writing, this debate continues with a recent multi-lab replication detecting a small, significant ego depletion effect (Dang et al., in press), and further pre-registered multi-lab attempts to replicate the effect in progress (Baumeister & Vohs, 2016b). Given the issues surrounding the replication crisis, participially regarding ego depletion, it is important that attempts are made to replicate findings that may impact future ego depletion research. The purpose of Chapter Four is therefore to conduct a replication of Chapter Three, with the goal of replicating, extending, and providing greater clarify as to the strength and direction of the results found.

In the present context, replication refers to improving and extending the method, often referred to as a direct replication (Schmidt, 2009). Core aspects of the study were therefore retained, namely the same independent and dependent variable operationalization and physical setting. However, opportunities were taken to make minor amendments to procedural details and independent variable stimuli, and one self-control task was replaced. As such, the present replication incorporated elements of both direct and conceptual replication (e.g., LeBel et al.,

2017). These changes were implemented to make methodological improvements based on procedural observations and the results of Chapter Three, while taking care not to deviate significantly from the original design facets.

First, a control group was added to extend the scope of the conclusions. Chapter Three conceptualized autonomous and controlled motivation as higher and lower quality motivation; nonetheless both experimental conditions received a dose of motivation. This distinction is important as although the quality of motivation may be lower, the quantity of motivation is nonetheless predicative of performance on simple tasks with a focus on outcomes (Cerasoli et al., 2014). The introduction of a group not receiving either high- or low-quality motivation will facilitate better understanding of the impact of autonomy-supportive and controlled regulation on the cortisol response and self-control by allowing comparison between the three conditions.

Second, the operationalization of the independent variables of autonomy-supportive and controlled regulation was adjusted. In Chapter Three, a relatively large effect size was observed as a result of a modest manipulation. Chapter Four attempted to increase the internalization of the Stroop task (Deci et al., 1994). This included using more autonomy-supportive or controlled language in the Stroop task instructions, as well as introducing two breaks within the modified Stroop task that were either a fixed length in the controlled regulation condition, or a self-selected length in the autonomy-supportive condition.

Third, the intrinsic motivation inventory (IMI; McAuley et al., 1989), administered as a manipulation check for the internalization of the modified Stroop was moved from the end of the study to directly after the modified Stroop. In Chapter Three, the manipulation check did not detect an experimental effect and it was felt the order of presentation may have contributed to this. In presenting the IMI at the end of the study, participants may have been unsure which task or tasks they were being asked about, or the internalization effect may have been diluted after performing several self-control tasks. Moving the manipulation check reduces the plausibility that these factors can be presented as alternative explanations. If the IMI is successful in detecting a difference between conditions, then it is likely that the placement of the IMI was a contributing factor in not detecting an effect. Conversely, if no effect is detected, then this raises concerns about the validity of the manipulation of motivation.

Finally, the third self-control task was replaced. It has been argued that exposing participants to the same self-control experiment twice can introduce confounding factors such as

cognitions or affective experiences (e.g., boredom) that may mask ego depletion effects (Chatzisarantis & Hagger, 2015). Although using identical self-control tasks is defendable (Lange, 2015), this modification increases the generalizability of the results, without compromising its scientific rigor. The third self-control task was therefore changed from a wall sit to a plank as it was felt this maintained a similar level of physical exertion where self-control remained a determinant of performance.

The results in Chapter Three partially confirmed the a priori hypotheses; namely, the effect of motivational manipulation on cortisol. However, the supplementary hypothesis regarding the motivational effect on self-control performance was rejected. Therefore, although the conceptual replication sought to extend the method, as previous hypotheses were constructed based on strong empirical support within the literature, the original hypotheses were not revised. The primary hypothesis was that participants manipulated into the controlled regulation condition would experience an increased cortisol response, while participants in the autonomy-supportive condition would experience a decreased cortisol response. Predicating the direction of the cortisol response in the control group, and based on previous work (Reeve & Tseng, 2011), it was speculated that there would be little change in cortisol across time in the control group.

The hypotheses regarding self-control performance were also retained. First, in line with the strength model of self-control, it was predicted that there would be no performance difference on the first self-control task, the modified Stroop. However, it was predicted there would be a performance difference on the second self-control task, the wall sit. Therefore, the second hypothesis was that participants in the autonomy-supportive condition would perform better on the wall sit that those in the controlled regulation condition. Although not guided by previous research, self-control performance in the neutral condition was expected to fall somewhere between that of the autonomous and controlled motivational groups. The final hypothesis regarding the ego depletion effect concerned performance on the third self-control task, the plank. Proponents of the strength model of self-control assert higher quantity motivation can increase self-control performance in the short term, however this effect will not persist over the longer term (Vohs et al., 2012). It was therefore predicted that on the third self-control task, the plank, there would be no discernible difference in performance between the autonomous and controlled conditions.

Finally, in Chapter Three an increase in cortisol coincided with improved wall sit performance, and post hoc analysis revealed an association. Due to the serendipitous nature of this finding, no specific predictions regarding replication of this effect were made, however instances of increases in cortisol coinciding with improved self-control performance would be explored statistically.

Method

Participants

The number of participants required to replicate the results of Chapter Three was determined by a power analysis conducted using G*power (Heinrich-Heine-Universität Düsseldorf, Düsseldorf). For the cortisol analysis, it was determined 57 participants would be required to detect the same effect size observed in Chapter Three with three experimental groups ($\alpha = .05$, $\beta = .80$, $\eta^2_p = .14$). The same power analysis for the observed wall sit power in Chapter Three was administered ($\alpha = .05$, $\beta = .80$, $\eta^2_p = .11$)[1], which determined 69 participants would be sufficient. Data were subsequently collected from 70 participants (Male = 18, Female = 52; M_{age} = 22, *SD* = 7) recruited using the same networks used in Chapter Three, with qualifying undergraduate students eligible to receive course credit in exchange for participation.

Measures

The measurement of cortisol in Chapter Four was identical to the procedure used in Chapter Three. However, the protocols for the following measures were adjusted.

Cognitive self-control. The modified Stroop task (Wallace & Baumeister, 2002) was employed as the cognitive measure of self-control. The operationalization aspects of the modified Stroop were identical to the version presented in Chapter Three, with two important differences. Firstly, two short pauses were incorporated at one-third intervals in all conditions. Secondly, the instructions for the modified Stroop were amended to emphasize either autonomy support or controlled regulation, with the neutral condition using the same instructions as Chapter Three.

Physical self-control. The procedure for the wall sit was identical to Chapter Three, however, the second wall sit was replaced with a plank. Starting from a prone position, and performed on a gym mat provided for comfort, the plank required the participant to raise their body off the ground by aligning the elbows directly under the shoulders and hands placed flat on the gym mat. Thus, the only points of contact with the ground are the elbows, forearms, hands

and toes, with the body forming a straight line from shoulders to heels. A standard sized sponge football was placed between the participant's torso and the floor, and they were asked to maintain contact during the task. The inclusion of the football was to maintain consistency of form between participants; specifically, to prevent participants elevating their hips, and therefore modifying the difficulty of the plank. Performance was determined by recording the time elapsed from the start of the task to failure to the nearest second. Failure was determined when the participant could no longer maintain the position, or when their form deviated from their start position, and they were unable to correct when verbally instructed within two seconds.

Manipulation check and covariates. Measures of mood, physical and mental exertion, intrinsic motivation and trait autonomy were retained from Chapter Three. In addition, current physical activity levels were measured as a potential covariate.

***Physical activity*.** The International Physical Activity Questionnaire Short Last 7-Day Self-Administered Format (IPAQ-S7S; Craig et al., 2003) was administered to control for individual differences in physical fitness on the physical tasks. The questionnaire measures the time spent physically active over the previous seven days, including work and leisure time. Activity is categorized into vigorous, moderate and walking, and includes a measure of sedentary time. Guidelines are provided for converting the intensity of physical activity into METs, with the converted scores then summed to provide a total MET score for physical activity. The IPAQ-S7S was chosen as it is most suited for use by young and middle-aged adults and has demonstrated test-retest reliability ($\rho = .75$), concurrent validity with the long form of the IPAQ ($\rho = .67$), and fair-to-moderate agreement with accelerometer data ($\rho = .30$; Craig et al., 2003; see Appendix Eleven).

Procedure

The procedure for Chapter Four followed the same format as for Chapter Three, including data collection and administration by the lead researcher. After obtaining informed consent and answering the screening questions (Appendix Twelve), participants provided a baseline measure of saliva collected via the passive drool method, and then completed the IPAQ-S7S and IAF.

There were three experimental conditions in the experiment; autonomy-supportive, controlled regulation and a neutral condition. The next phase of the study followed the protocols used in Chapter Three, namely participants in the autonomy-supportive and controlled regulation

condition were offered a choice of cognitive task. The options were (a) The Mental Distraction Game; (b) A Game of Accuracy; c) Ignore Your Impulses, and (d) Cognitive Response Latency Test, with the experimental manipulation following the same procedure as Chapter Three. The neutral group were not exposed to any manipulation, and moved directly onto the next phase of the experiment, the modified Stroop

Although the structure of the modified Stroop was similar to Chapter Three, there were some minor amendments designed to increase the salience of the motivational manipulation. Internalization of the Stroop task followed established protocols designed to manipulate the internalization of an uninteresting computer task (Deci et al., 1994; see Appendix Thirteen). The instructions to the tasks between conditions employed language designed to enhance the motivational salience. In the autonomy-supportive condition, low controlling language was used, in contrast to high-controlling language in the controlled regulation condition. The neutral condition used neutral language. Examples are "we would like you to press" (vs "you must press"); "If you are willing to participate, please begin the activity by pressing the space bar" (vs "you should start the experiment by pressing the space bar"). After participants had read the instructions, they completed a short practice of the modified Stroop that was identical to Chapter Three and were then given the opportunity to ask any questions. They then proceeded with the modified Stroop.

The experimental manipulation was further enhanced by incorporating two pauses in the modified Stroop. At the commencement of the pause, a message was displayed that was relevant to the experimental manipulation. In the autonomy-supportive condition, participants were presented with the message "You now have a short break, press any key to continue". In the controlled regulation condition, participants were told "You now have a short break, the experiment will recommence shortly", with the break fixed at 10,000ms. In the neutral condition, the message read "You now have a short break, the experiment will recommence shortly, or press any key to continue". In this condition, participants could press any key to recommence the study, or if no key were pressed, the experiment restarted after 10,000ms.

Once participants had completed the modified Stroop, they indicated how much mental exertion the task required via the Borg's single-item CR-10 scale (Borg, 1998), and were asked to complete the IMI. A procedural detail was altered at this point, with the IMI moved from the

end of the experiment as with Chapter Three, to directly after the modified Stroop. The next phase of the experiment mirrored the procedure employed in Chapter Three. Participants completed a wall sit, followed by indicating how much physical exertion the task required, via the Borg's single-item CR-10 scale (Borg, 1998); provided a second saliva sample; and perform the congruent Stroop, followed by participants indicating mental exertion via the Borg's single-item CR-10 scale (Borg, 1998).

In the final alteration to the procedure employed in Chapter Three, participants were asked to perform a plank. Upon completion of the task, participants then indicated physical exertion on the task, via the Borg's single-item CR-10 scale (Borg, 1998). Finally, participants completed the BMIS, provided a final sample of saliva and were debriefed as to the true nature of the study and thanked for their participation. A graphical representation of the procedural differences of Chapters Three and Four is shown in Figure 4.1.

Data Analysis

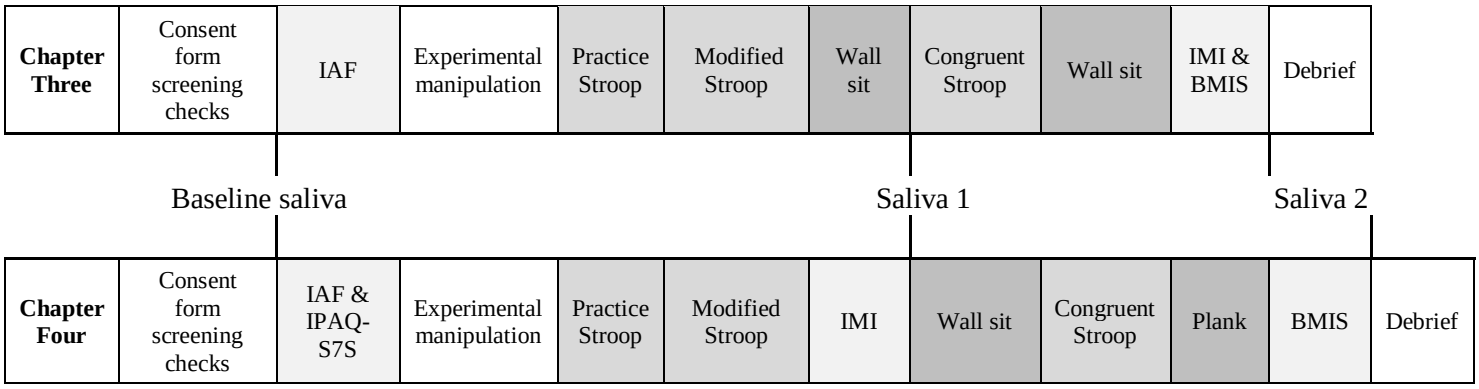
The procedure for cortisol and Stroop data analysis followed the same procedure identified in Chapter Three. For the plank and the wall sit, main analysis was conducted using a one-way ANOVA. The IPAQ-S7S was added to the list of covariates that were checked as predictors of physical self-control performance in Chapter Three.

Results

Data Checks

All data were normally distributed and had homogenous variances, aside from the distribution of the cortisol samples. For the cortisol analysis, the same procedure for treatment of data as Chapter Three was used. This resulted in removal of two participants; one reported waking less than 60 minutes before the test, and one whose baseline cortisol level was greater than three standard deviations above the mean. Despite the removal of these outliers, Shapiro-Wilk tests indicated that cortisol at baseline (W (20) = .83, p = .003), timepoint 1 (W (23) = .89, p = .016) and T2 (W (23) = .87, p = .006) was not normally distributed, and therefore data were log-10 transformed. This corrected the distribution at all timepoints and conditions, except for the autonomy-supportive condition at timepoint 1. Examination of the boxplot revealed two outliers at this timepoint for participants 22 and 40. Removal of this data normalized Shapiro-Wilkes distribution to within acceptable levels (W (20) = .93, p = .14). Further examination of

Figure 4.1. *Experimental procedure for Chapters Three and Four*



the log transformed data revealed equal covariance across groups (Box's $M = 28.63$, $p = .009$), Mauchly's test indicated sphericity had not been violated ($\chi^2(2) = .96$, $p = .325$) and Levine's test of homogeneity of variance between groups was maintained (F (2,57) = 2.50, $p = .091$). Means, standard deviations, bivariate correlations and Cronbach alpha levels for the study variables are reported in Table 4.1. There were no significant differences between conditions for mood (BMIS), trait autonomy (IAF), subjective effort on the tasks (Borg DR-10 scale), or physical activity levels. The IMI was administered as a check to see if the experimental manipulation was successful in internalizing an uninteresting computer task. A one-way ANOVA revealed a significant difference between groups for the subscales of interest/enjoyment (F (67,2) = 3.44, $p = .038$, $\eta_p^2 = .07$) and value/usefulness (F (67,2) = 4.83, $p = .011$, $\eta_p^2 = .13$), but not for perceived choice (F (67,2) = 0.89, $p = .415$, $\eta_p^2 = .03$). Post hoc Tukey HSD revealed the effect of interest/enjoyment and value/usefulness were driven by significant differences between the autonomy-supportive and neutral conditions ($p = .037$; $d = .69$ and $p = .008$, $d = .92$ respectively). Furthermore, the mean scores for autonomy-supportive were higher than for the controlled regulation condition across all subscales, although they did not reach statistical significance. It is important to note that the interest/enjoyment subscale is considered the self-report measure of intrinsic motivation, and therefore the internalization of the modified Stroop can be considered successful (Deci et al., 1994). Finally, validating the lower effort required on the congruent versus modified Stroop, there was a significant difference in perceived cognitive effort measured via Borg-CR10 scales (t (70) = 7.36, $p = <.001$).

Cortisol

Cortisol analysis was performed using the log-10 transformed scores. Prior to analysis, the same potential covariates used in Chapter Three that could affect the cortisol response were examined. To test if the potential covariates affected the cortisol response, a regression model was constructed, with cortisol at timepoint 1 as the dependent variable, and baseline cortisol and the potential covariates included as predictor variables. The multilinear regression model was significant (F (7, 51) = 15.71, $p < .001$, $\eta_p^2 = .68$); however, this was driven by baseline cortisol significantly predicting cortisol at timepoint 1 ($t = 1.92$, $B = .83$, $p < .001$), with none of the other covariates emerging as statistically significant predictors, thus excluding them from the main model.

Table 4.1.

Data Checks Between the Autonomy-Supportive, Controlled Regulation and Neutral conditions

	Autonomous	Controlled	Neutral											
	M(*SD*)	*M*(*SD*)	M(SD)	1	2	3	4	5	6	7	8	9	10	11	α
1, SC (IAF)	3.77(.44)	3.72(.33)	3.88(.62)												.68
2, StC (IAF)	2.78(.52)	2.63(.71)	2.66(.63)	-.068											.58
3, IT (IAF)	3.93(.76)	3.75(.66)	3.79(.68)	.216	.024										.81
4, I/E (IMI)	3.16(.93)	3.01(.75)	2.53(.89)	.235	-.046	.366**									.94
5, V/U (IMI)	3.42(.61)	3.12(.76)	2.76(.81)	.16	-.099	.183	.634**								.91
6, PC(IMI)	4.47(.63)	4.41(.67)	4.21(.76)	.108	-.109	.183	.475**	.535**							.87
7, BMIS	3.14(.31)	3.06(.34)	2.99(.42)	.223	-.057	.089	.340**	.182	.283*						.79
8, CR-10M 1	5.38(1.93)	4.74(1.60)	5.13(2.05)	.031	.19	-.093	.008	-.018	-.119	-.014					-
9, CR-10P 1	5.92(2.30)	5.70(1.77)	5.70(2.68)	-.043	-.075	.046	.096	.237*	.236*	.083	.271*				-
10 CR-10M 2	3.10(1.99)	3.00(1.90)	3.41(2.22)	.187	-.035	.079	.056	.083	-.041	.210	.374**	.418**			-
11, CR-10P 2	6.33(2.53)	5.96(2.08)	6.65(2.42)	-.057	.109	-.004	.035	.08	.037	-.003	.410**	.740**	.411**		-
12, IPAQ-S7S	3728(1978)	3539(3516)	2731(1710)	-.001	-.215	.128	-.057	.047	.091	.128	.097	.128	-.075	-.031	-

*$p < .05$ **$p < .01$; SC = self-congruence; IAF = index of autonomous functioning; StC = susceptibility to control; IT = interest taking; I/E = interest/enjoyment; IMI = intrinsic motivation inventory; V/U = value/usefulness; PC = perceived choice; BMIS = brief mood introspection scale; CR-10 = Borg single-item CR-10 scale; IPAQ-S7S = international physical activity questionnaire last 7-day self-administered format

Subsequently, a one-way repeated measures ANOVA was constructed, with experimental condition as the independent variable and cortisol as the repeated measure. The within subject analysis revealed a significant effect of time on cortisol (Wilks' Lambda F (2,114) = 3.18, p = .045, η_p^2 = .05), and a non-significant between subject effect (F (1, 57) = 0.85, p = .431, η_p^2 = .03). Further analysis revealed a significant linear within subject contrast (F (2, 57) = 4.45, p = .016, η_p^2 = .14). As can be seen in Figure 4.2, there was a non-significant deviation from baseline in either the autonomy-supportive or controlled regulation conditions. However, there was a significant linear decline in cortisol in the control condition. It is important to note that the inclusion of the data discarded during the data check did not affect the significance of the within-contrasts linear effect (F (2, 60) = 3.49, p = .037, η_p^2 = .10), and neither did the inclusion of the waking-to-baseline saliva time, which emerged as a significant covariate in Chapter Three (F (2, 56) = 4.46, p = .016, η_p^2 = .14). The descriptive statistics for the back transformed cortisol data are shown in Table 4.2.

Self-Control Performance

Modified Stroop. A full list of congruent and modified Stroop dependent variables is listed in Table 4.3. As predicted, there was no significant difference in performance between groups on the modified Stroop task.

Wall sit. First, potential covariates of wall sit performance were checked for associations with the total time recorded for the wall sit. Neither gender, age, current physical activity levels, physical activity engaged in during the last 24 hours, or whether they had performed a wall sit before were associated with wall sit performance, and therefore these were excluded from further analysis. A one-way ANOVA revealed a performance difference between experimental conditions on wall sit performance that fell just short of conventional statistical significance (F (67,2) = 2.88, p = .063, η_p^2 = .08). A graphical representation can be seen in Figure 4.3. Post hoc Tukey HSD test revealed that participants in the autonomy-supportive condition performed better than those in the controlled regulation condition, with the difference again falling just short of conventional statistical significance (114 seconds v 78 seconds respectively, p = .055). There was no significant difference between the neutral condition, and the autonomy-supportive or controlled regulation conditions (p = .734 and p = .258 respectively).

Plank. First potential covariates for plank performance were checked using the same predictors as the wall sit. Neither age, physical activity engaged in during the last 24 hours or

Figure 4.2.

Repeated measures ANOVA showing cortisol response between conditions with standard error bars

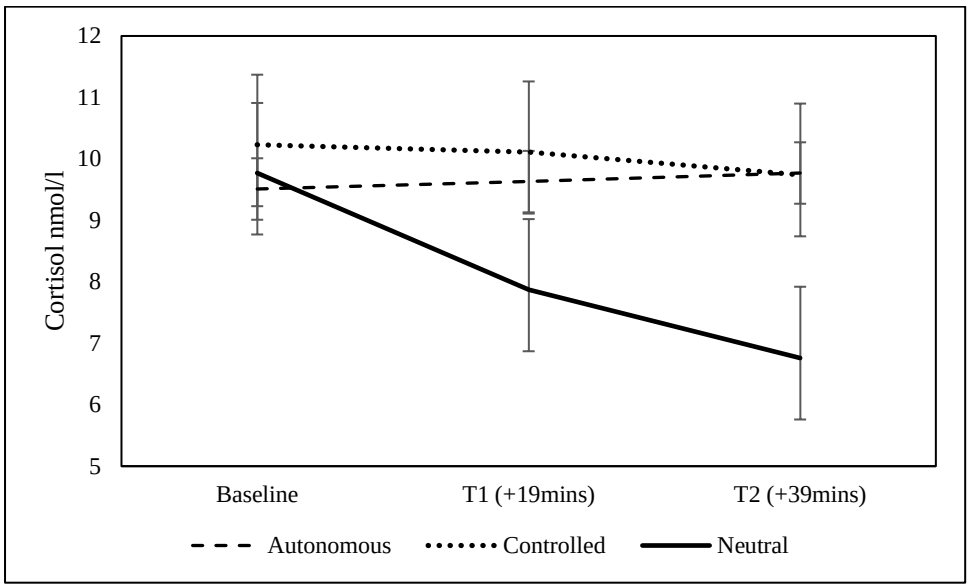


Table 4.2.

Descriptive Statistics for Back Transformed Cortisol Data

	Autonomy-supportive		Controlled regulation		Neutral	
Cortisol	*M (SE)*	95% CI	*M (SE)*	95% CI	*M (SE)*	95% CI
Baseline	9.51(1.14)	[7.37, 12.24]	10.23(1.14)	[8.04, 13.34]	9.77(1.14)	[7.58, 12.59]
Timepoint 1	9.63(1.15)	[7.29, 12.70]	10.11(1.15)	[7.66, 13.37]	7.87(1.15)	[5.96, 10.40]
Timepoint 2	9.77(1.16)	[7.21, 13.27]	9.74(1.16)	[7.19, 13.24]	6.76(1.16)	[4.98, 13.24]

Table 4.3.

Dependent Variables and Inferential Statistics for the Stroop Tasks

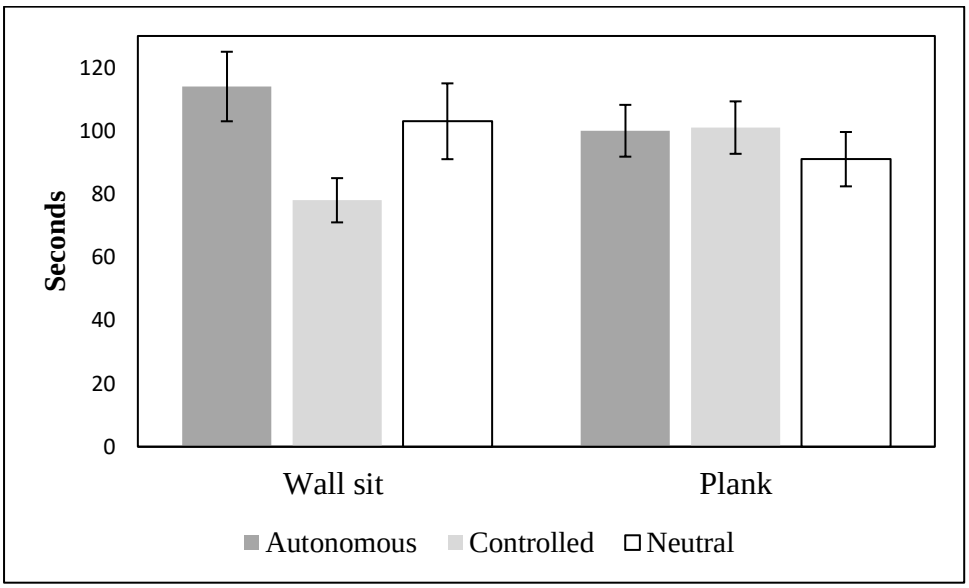
	Autonomy-supportive		Controlled regulation		Neutral		N		
Modified Stroop	N *M(SD)*	RT *M(SD)*	N *M(SD)*	RT *M(SD)*	N *M(SD)*	RT *M(SD)*	*F* =	*p* =	η_p^2 =
Correct Responses	278(40)	689(48)	288(55)	691(42)	262(57)	703(59)	1.48	.236	.042
Incorrect responses	36(27)	746(67)	691(42)	732(79)	41(50)	736(58)	.182	.834	.005
No Response	45(29)	-	40(26)	-	57(38)	-	1.81	.172	.051
Congruent Stroop									
Correct Responses	345(11)	558(52)	347(10)	551(46)	344(7)	554(70)	.457	.635	.013
Incorrect responses	12(9)	546(64)	11(9)	528(74)	13(7)	531(100)	.211	.810	.006
No Response	3(5)	-	2(2)	-	3(3)	-	.478	.622	.014

Notes: N=number of responses RT=reaction time in milliseconds

.

Figure 4.3.

Wall sit and plank mean times between conditions with standard error bars



whether they had performed a plank before were associated with wall sit performance. However, gender (r = -.48, p < .001) and current physical activity levels (r = .33, p = .005) were significantly associated with performance on the plank and were therefore included as covariates in the main analysis. A one-way ANCOVA was subsequently constructed with plank time as the dependent variable, experimental condition as the independent variable, and gender and current physical activity level as covariates. However, there were no significant differences between autonomy-supportive, neutral and controlled regulation plank times (106 seconds v 92 seconds v 92 seconds respectively; F (64,4) = 0.41, p = .664, η_p^2 = .03).

Effects of Cortisol on Self-Control Performance

As there was not a significant increased cortisol response across any experimental conditions, it precluded constructing models that would test for associations between increased cortisol and improved self-control performance.

Discussion

The aim of this study was to replicate and extend Chapter Three. The primary hypothesis sought to reproduce the cortisol response observed in Chapter Three, namely, an attenuated cortisol response in the autonomy-supportive condition, and an increased cortisol response in the controlled regulation experimental conditions. However, this hypothesis was not supported, with no significant differences between autonomy-supportive and controlled regulation conditions. However, a significant linear decline in cortisol was observed in the neutral condition. In addition, both self-control hypotheses were supported; greater wall sit performance was observed in the autonomy-supportive condition compared with the controlled regulation condition, however this performance difference between conditions disappeared on the final self-control task. Contrasting the results of Chapter Three, the results of this study therefore offer further support for the beneficial effects of autonomy-supportive motivation in improving self-control performance in the short term. Furthermore, it supports the idea that motivational effects on self-control are time-limited, and do not persist over multiple self-control trials (Vohs et al., 2012; Graham et al., 2014).

The results of Chapter Four did not replicate the cortisol response observed between conditions in Chapter Three. Specifically, an increased cortisol response in the controlled regulation condition, and an attenuated response in the autonomy-supportive condition was not observed. However, there was a significant effect of cortisol over time, which upon further analysis was driven by a linear decline in cortisol in the neutral condition. The inclusion of a neutral condition is a useful tool for interpreting cortisol changes as it allows

the interpretation of motivational effects compared with the neutral condition. For example, a similar study including controlled, autonomous and neutral motivational conditions reported a non-significant deviation from baseline in a neutral condition, with an increase in cortisol in the controlled condition, and a decrease in the autonomous (Reeve & Tseng, 2011). The decline in cortisol in the neutral condition represents an important finding; it suggests that the modified Stroop may not have been sufficiently stressful to provoke a cortisol response in the present population. This interpretation is supported by the lack of an increase in cortisol in either the autonomy-supportive or controlled regulation conditions. The modified Stroop is a moderately stressful task (Renaud & Blondin, 1997) and has been demonstrated to provoke a cortisol response similar to that observed in the Trier Social Stress Test (Kirschbaum, Ebrecht & Hellhammer, 2001). However, other studies examining cortisol response to the Stroop task have found smaller effects (e.g., Bakke et al., 2004), and therefore the reliability of the modified Stroop in provoking a cortisol response may be questionable. This is important; if the stressor task does not produce a significant stress response, attempts to moderate and measure high- and low-quality motivational processes are likely to be compromised. Furthermore, individual differences in the perception of threat and coping resources (Carver & Vargas, 2011) may have influenced results, for example, if the participants had previous experience of the Stroop task. In conclusion, given the weight of evidence provided by Chapter Two and Three supporting the idea that high-quality motivation attenuates the cortisol effect, it would be premature to draw too much significance from the present finding. Moreover, the reliability of the modified Stroop in provoking a cortisol response, and individual differences in coping resources may provide alternative explanations for the lack of support for the primary hypothesis.

The second hypothesis concerned self-control performance. In line with predictions, participants in the autonomy-supportive condition performed significantly better on the wall sit than those in the controlled regulation condition, with no difference in performance between either of those conditions and the neutral condition. These results support several studies that have found a positive effect of autonomous, and a negative effect of controlled motivation on self-control performance (Muraven, 2008; Muraven, et al., 2008; Muraven & Slessareva, 2003). Furthermore, the results of this study replicate the effect of autonomy-supportive versus controlling environments on modified Stroop and physical self-control performance (Graham et al., 2014). Thus, both self-control related hypotheses were supported, and offer further support for the benefits of autonomous motivation in improving

performance during self-control task, even if such an effect does not persist over multiple self-control tasks.

The results of the present study therefore contradict Chapter Three, where participants in the controlled regulation condition outperformed autonomy-supported participants on the wall sit tasks. Although the results of Chapter Three cannot be dismissed, they are perhaps indicative of the inconsistent results found elsewhere in the self-control literature (Carter & McCullough, 2014; Carter et al., 2015; Hagger et al., 2016). However, although this may be a symptom of the presenting findings, examination of the operationalization and results of Chapter Three and Four is warranted. First, the raw wall sit times between conditions and studies was compared. In the autonomy-supportive condition, the times for wall sit one were 124 seconds in Chapter Three, and 114 seconds in Chapter Four, representing a degree of reliability between studies. However, there was a large difference between studies in the controlled regulation condition; 158 seconds in Chapter Three, versus 78 seconds in Chapter Four. This is obviously a large, interesting and potentially important finding. As this was a direct replication, with only the operationalization of the IV altered prior to the administration of the wall sit, there are few plausible explanations for a discrepancy of this size. As speculated in Chapter Three, it is possible individual differences in strength is a factor, or that the increased cortisol in the controlled regulation condition facilitated a greater performance on the wall sit. Examining plausible explanations for this unexpected finding, it was speculated the cortisol response in the controlled regulation condition may have energized wall sit performance, and post hoc statistical analysis provided some support for this proposition. In the present chapter, there was no corroborating evidence for an energizing effect. However, it should be noted the lack of a cortisol response in any condition precluded investigating this effect.

It was also speculated in Chapter Three that individual differences in strength may have confounded results. In Chapter Three, participants in the controlled regulation condition maintained a similar level of performance over the autonomy-supportive group across both wall sit tasks. Moreover, on the second wall sit, although performance in the controlled regulation condition declined, it declined to a similar degree in the autonomous condition. This indicates that the confounding variable was evident across wall sit one and two. Furthermore, physical activity was not controlled for as rigorously in Chapter Three as in the present chapter, thus this could not be eliminated as an alternative explanation. In conclusion, individual differences in strength appears the most likely explanation for the greater wall sit

performance in Chapter Three, with both corroborating and little contradictory evidence. However, as the energizing effect of cortisol as an explanation could not be eliminated, further research may be the only way to satisfactorily address this question.

Our final hypothesis concerned persistence of motivational effects on self-control performance. Specifically, it was predicted motivational effects on self-control performance would dissipate on the plank due to prior self-control depletion. The results of this chapter supported this hypothesis; there was no difference in performance between experimental conditions on the plank. This result contrasts the result of Chapter Three, where there was a significant difference in performance between the first and second wall sit. The present results replicate the wider literature from which the hypothesis was drawn (Graham et al., 2014; Vohs et al., 2012). Furthermore, as previously discussed, if strength differences between groups in Chapter Three confounded those results, then the persistence of a difference between conditions on self-control performance is potentially unreliable. It should be noted that the plank has not been previously employed as a measure of self-control performance or ego depletion. This contrasts with the wall sit that has been validated in self-control research that has explicitly measured the ego depletion effect (Boat & Taylor, 2017). This point notwithstanding, in the context of repeated measures of self-control, results appear to better align with the wider self-control literature rather than the results of Chapter Three and offers implicit support for a limited resources account of self-control.

A final point of discussion relating to physical self-control performance was the emergence of gender as a covariate. Gender was associated with performance on the second physical task, but not the first task in Chapters Three and Four, with males outperforming female participants. There is little evidence that gender has been considered as a factor in previous research examining performance on multiple self-control tasks (Graham et al., 2014; Vohs et al., 2012). It is tempting to conclude that males performed better on multiple physical self-control tasks due to group differences in strength; however, this does not explain why there was no evidence of increased performance between gender on the first physical task. Thus, it may be that there was a gender difference in physical endurance or recovery within the present physical tasks. The lack of evidence supporting gender effects leaves this occurrence unexplained and warrants consideration in future research examining multiple self-control tasks with an evaluative physical element.

Limitations and Future Directions

The major limitations of Chapter Three were largely addressed in Chapter Four. Minor protocols were improved; a control group was introduced, and a physical activity questionnaire was administered to control for physical differences on the physical tasks. However, a limitation is the modified Stroop task may not have been stressful enough to reliably detect attempts to moderate the cortisol response. Future research may wish to investigate the reliability of the modified Stroop as a stressor task and consider alternative methods of investigating the potential effects of cortisol on self-control performance, which may help to clarify this inconsistency.

Conclusion

The results of the present research offered support for the self-control hypotheses; however, the cortisol results observed in Chapter Three were not replicated. There was a significant cortisol difference between groups over time; however, this was drive by a linear decline in cortisol in the neutral condition, with little differences observed in the autonomy-supportive or controlled regulation conditions. This result suggests the modified Stroop may not have been an adequate stressor in the present study. Self-control performance in the present chapter was in line with expectations, with participants in the autonomy-supportive condition demonstrating improved self-control performance compared with participants in the controlled regulation condition. Supporting the final hypothesis, the difference in self-control performance between groups did not persist to the final self-control task. This suggests that motivational interventions may lead to adaptive self-control performance, but perhaps only in the absence of an increased cortisol response.

Although the results observed across Chapters Three and Four were not conclusive, support was found to varying degrees for all hypotheses. Specifically, autonomous motivation led to a lower stress response in Chapter Three and improved self-control performance in Chapter Four. Research into the motivational effects of higher quality motivation on physiological mechanisms remains in its infancy, and further work – particularly including performance measures - is encouraged.

Chapter Five

Study Four

Health-Related Motivational and Behavioural Processes Associated with DNA Methylation of *TNF*

Abstract

Epigenetics has been described as one of the most exciting areas of contemporary biology, and research has begun to explore whether epigenetic modifications are influenced by psychological processes. The present research explored the associations of health-related motivation and behaviour with the DNA methylation of tumor necrosis factor (*TNF*) gene. Participants (N = 88) completed questionnaires examining engagement with health-related behaviour (i.e., physical activity, diet and smoking) and health-related motivation from the perspective of self-determination theory. They also provided a capillary blood sample for DNA extraction and analysis of four CpG sites via bisulfite conversion within Exon 1 of *TNF*. Path analysis supported the hypothesis regarding direct effects. Supporting the idea that high quality motivation is most important for health-related behaviour and outcomes, health-related autonomous motivation was weakly but positively associated with *TNF* methylation (β =.18, p =.08); however, health-related introjected regulation was not associated with *TNF* methylation (β =.13, p =.23). Indirect effects were identified in a subsequent step; autonomous motivation was positively associated with fruit consumption (β = .29, p = .004), negatively associated with smoking (β = -.22, p =.03) but not associated with physical activity (β = .10, p = .34). Moreover, *TNF* methylation was positively associated with lifetime physical activity (β = .18, p =.08) and negatively associated with smoking (β =-.23, p =.03). Direct effects of autonomous motivation on *TNF* methylation did not persist when these indirect effects were included (β =.09, p =.43). Results support the idea that autonomous motivation is associated with DNA methylation of the *TNF* promoter, albeit indirectly through tobacco consumption.

Keywords: Epigenetic, physical activity, health, self-determination theory

Introduction

Epigenetics has been described as one of the most exciting areas of contemporary biology (Bird, 2007). Epigenetics is the study of heritable changes in the phenotype that do not alter the sequence of DNA (Carey, 2012). Since the inception of epigenetic research, it has been broadly focused on the environmental impacts on genetic modifications. However, recent investigations have begun to extend the idea that psychological processes are implicated in as an epigenetic mechanism (e.g., Cole 2013). Research in this area has tended to focus on the DNA methylation of candidate genes associated with inflammatory and immune functioning, because of the implications for developmental and psychopathological processes (Jones et al., 2018). For example, a recent review examined the pathogenesis of disease in relation to physical activity and the methylation of inflammation-related epigenetic markers (Horsburgh et al., 2015). Horsburgh and colleagues suggested the inflammatory response to acute physical activity was potentially a regulatorily mechanism for changes in DNA methylation, with the review highlighting implications for healthy and diseased populations. The present research extends this line of investigation by focusing on regulatory motivational process involved in physical activity, health-related behaviour, and the methylation of the *TNF* gene promoter associated with the expression of circulatory TNFα.

TNFα is a proinflammatory cytokine that plays a prominent role in the body's immunomodulatory system. In response to inflammation, TNFα is stimulated and regulates the accumulation of macrophages and neutrophils to kill pathogens at the site of inflammation (Vassalli, 1992). It helps to induce fever and protect against viruses (Tortora & Derrickson, 2017), as well as other important functions in the body, such as assisting the regulation of glucose and fat metabolism (Hotamisligil & Spiegelman, 1994). However elevated levels of circulatory TNFα have been associated with several noncommunicable diseases, including type one diabetes (Arroyo-Jousse et al., 2016), rheumatoid arthritis (Maxwell et al., 2008), and Crohn's disease (Van Deventer, 1997). Furthermore, methylation at the *TNF* gene has been identified as a limiting risk factor in some disease states (for example obesity; Campión, et al., 2009; Milagro et al., 2011). In summary, regulating circularity levels of TNFα is important in maintaining the body's ability to deal with inflammation, while not compromising susceptibility to disease. For example, Crohn's disease is often managed using medication that reduces circulatory levels of TNFα; however, this can leave the patient at risk of increased susceptibility to infection (National Health

Service, 2018). Therefore, the identification of modifiable factors that can positively influence methylation of the *TNF* gene represents a worthwhile scientific endeavour.

To a small degree, psychological experiences have been investigated as a possible source of epigenetic changes because it offers significant potential to enhance understanding of how everyday states shape human development. For example, subjective experiences of social isolation, but not objective social isolation, are associated with the up-regulation of pro-inflammatory genes, and down-regulation of anti-viral and antibody regulated genes (Cole et al., 2007). Furthermore, stress, post-traumatic stress disorder, and self-control have also been implicated as psychological mechanisms associated with DNA methylation (Lam et al., 2013; Miller et al., 2015; Smith et al., 2011). This line of investigation suggests that subjective psychological states may play a prominent role in epigenetic modifications. The focus of the present paper is to propose and explore a novel area of psychological influence on DNA methylation of *TNF*, namely human motivation.

Traditionally, human motivation has been conceptualized as a unidimensional, linear process concerned with the direction and energization of behaviour towards positive stimuli (Elliott, 2006). Within this paradigm, motivation has been empirically linked to physiological changes; most notably cardiovascular responses (Richter et al., 2016). For example, increases in cardiovascular reactivity occur as cognitive effort increases, provided success is deemed possible and effort is worthwhile (Richter et al., 2008). Complementing this perspective, multidimensional motivation theories differentiate not only the amount of motivation, but also the quality of motivation. When considering health-related choices, many healthy behaviours are driven by extrinsic motives, rather than an inherent love, enjoyment or interest in the activity. For example, there is little pleasure in refraining from smoking or resisting a large slice of cake, but many people choose to do so because being a healthy person is important, or to avoid guilt after succumbing to the temptation. A motivation theory that captures this differentiation is organismic integration theory (OIT; Ryan & Deci 2017). Grounded in the larger self-determination meta-theory, OIT proposes extrinsic motives vary in self-determination and quality, and can be placed on a continuum, with behaviour that is pursued for more autonomous, internalized reasons contrasted with behaviour that is underpinned by controlled motivation (Ryan & Connell, 1989). When a behaviour is fully endorsed and aligned with an individual's core values or beliefs, it is based on integrated motivation. Identified motivation reflects pursuing a behaviour because it is personally meaningful, although not necessarily fully aligned with all other aspects of the self (Ryan &

Deci, 2017). Although integrated and identified motivation are classified as extrinsic in origin, they nonetheless represent behaviour that is internalized and therefore autonomously regulated (Ryan & Deci, 2000b). Introjected motivation represents the shift on the continuum from autonomous to controlled motivation and encompasses behaviour that is controlled internally, such as a desire to avoid guilt or seek approval (Ryan, 1982). Finally, behaviour can be externally regulated, such as contingent rewards or punishments (Ryan & Deci, 2000a). Although many behaviours are externally regulated, such as working in an unsatisfying job for the salary, motivation to engage in healthy behaviour is rarely externally regulated for adults and is therefore less relevant in the present context.

The degree to which behavioural regulation is internalized has important health implications. Behaviour that is underpinned by higher quality integrated and identified reasons is associated with improved outcomes in health care and health promotion settings (e.g., Ng et al., 2012). Conversely, when behaviour is undertaken for lower quality, controlled reasons, it not only yields less likelihood of long-term adherence, but it may also lead to psychological dysregulation (Deci & Ryan, 2008). Furthermore, there is emerging evidence to suggest that this motivational distinction can help predict downstream physiological responses. For example, environments that facilitate greater autonomous functioning are linked to adaptive social stress responses in the endocrine system (Reeve & Tseng, 2011). In addition, high quality intrinsic motivation is associated with adaptive dopaminergic processes, such as greater dopamine D2-receptor availability (de Manzano et al., 2013; see also Di Domenico & Ryan, 2017). This study aimed to extend this broad motivation-physiology paradigm to epigenetic modifications.

Based on the above review, this study will adopt an exploratory approach to investigate integrated, identified and introjected motivations for healthy behaviour as potential correlates of *TNF* methylation. Exploratory research represents an important part of most research programs, yielding the opportunity to uncover serendipitous occurrences and construct complicated multivariate structures (Ledgerwood, Soderberg & Sparks, 2017). Furthermore, this study aimed to establish whether any observed associations persist after accounting for indirect effects via lifestyle factors, specifically physical activity, diet, alcohol consumption and smoking. These lifestyle choices have been linked to wider DNA methylation (Breitling, Yang, Korn, Burwinkel & Brenner, 2011), methylation at the *TNF* promoter (Bollati et al., 2014; Shaw et al., 2014), and could, therefore, plausibly explain any observed motivational effects (i.e., indirect effects exist). Alternatively, motivation may have

direct associations with epigenetic modifications that exist over and above energizing behavioural choice. The former 'indirect effects' conclusion would provide evidence for a motivational emphasis in attempts to modify healthy behaviour and subsequent epigenetic states. The latter 'direct effects' conclusion would suggest the need to investigate currently unknown motivational processes influencing gene expression.

Method

Participants

Ethical clearance was granted by the Loughborough University Approvals (Human Participants) Sub-Committee. A power analysis to detect a coefficient of determination greater than 5% suggested that it would require 88 participants to detect this size of effect ($\alpha = .10$, $\beta = .80$, $r^2 > .05$). A generous alpha level was adopted given the exploratory nature of the study. Data were subsequently collected from 54 women and 34 men recruited from a university campus opportunity sample between May and August 2016, with participants receiving no compensation for their time. The sample was recruited via advertising on a university website, as well as posters in the local area. All participants provided written informed consent prior to taking part. Socioeconomic status of the cohort was relatively affluent (range: 1 = least deprived to 10 = most deprived; $M = 3.90$, $SD = 2.25$). The participants' ages ranged between 19 and 74 ($M_{age} = 32.28$ years, $SD = 14.01$). The population was healthy in several measurable aspects, including a mean BMI of 24.60 ($SD = 4.24$); mean current weekly leisure time physical activity (LTPA) of 5.63 hours ($SD = 5.32$), and a mean Dietary Quality Score (DQS) of 9.68 ($SD = 1.47$) out of a maximum score of 12. In addition, 64 participants reported never having smoked, with 78 participants consuming an average of 2 units of alcohol or less per day.

Measures

Motivation for healthy behaviour. A hybrid motivation questionnaire grounded in OIT was designed to measure the degree to which healthy behaviours were underpinned by different motivational regulations. Introjected and identified motivation were measured using six items from the Exercise Self-Regulation Questionnaire (Ryan & Connell, 1989). This questionnaire does not include a subscale to measure integrated motivation, hence this type of motivation was measured using four items drawn from the Behavioural Regulation in Exercise Questionnaire (version 3; Markland & Tobin, 2004). As the items contained in both questionnaires were originally developed for measuring exercise behaviour, they were adapted to measure the broader construct of health behaviour. For example, the original item

"I consider exercise a fundamental part of who I am" was changed to "I consider healthy behaviour a fundamental part of who I am". Responses were measured on a 5-point likert scale from 0 (*not true for me*) to 4 (*very true for me*). The subscales of introjected, identified and integrated motivation have all demonstrated acceptable reliability in previous research (α > .73; González-Cutre et al., 2014; Silva et al., 2011)

Diet. The DQS (Toft, Kristoffersen, Lau, Borch-Johnsen, & Jørgensen, 2007) is a four-item questionnaire designed as a brief instrument to assess diet. Higher DQS scores are negatively associated with cholesterol, triglyceride, low-density lipoprotein-cholesterol, homocysteine and risk of heart disease, and positively correlated with a longer dietary assessment (i.e., 198-item Food Frequency Questionnaire; Toft et al., 2007). The four items measure intake of vegetables, fruit, fish and fat, respectively, with a range of 1-3 for each item. For example, the item assessing fruit intake was followed by the response choice of either "3 pieces/day", "more than 3 pieces/week and less than 2 pieces/day" and "less than 2 pieces/week", with a score of 3, 2 or 1 attributed, respectively. Scores were then summed to give a range of 4-12, with a higher score indicating a higher quality diet.

Physical activity. LTPA and occupational physical activity was assessed using the Modifiable Activity Questionnaire (MAQ; Kriska et al., 1990). The questionnaire provides formulae for calculating the average number of hours engaged in leisure time and occupational activity during past year and lifetime, with leisure time physical activity also recorded over the previous week. The MAQ provides protocols for the conversion of moderate and hard occupational physical activity to metabolic equivalents (METs; total hours multiplied by 4 and 7 respectively). For consistency, the LTPA scores were also converted to METS using established MET activity equivalents (Ainsworth et al., 2000). Test-retest reliability of the MAQ for historical physical activity is good, with past year (r = .89) and lifetime (r = .94) strongly correlated with measures taken up to three weeks previously. The MAQ also has good validity between activity monitor counts and weekly physical activity (r = .66; Kriska et al., 1990), and compares favourably with alternative questionnaires measuring short and long-term physical activity (De Vera, 2006).

Tobacco and alcohol consumption. Participants were asked about their level of alcohol and tobacco consumption using a questionnaire based on an assessment tool used by the UK National Health Service (Lifestyle Questionnaire, n.d). Tobacco consumption was measured on an 8-point scale ranging from 1 (*Never ever smoked*), to 8 (*more than 40 a day*).

Alcohol consumption was measured in a 6-point scale ranging from 1 (*I never drink alcohol*") to 6 (*I drink more than 9 units a day*).

Procedure

Participants were asked to provide written informed consent and then completed the self-report questionnaires (see Appendix Fourteen). In accordance with the standard instructions, the MAQ questions were read aloud for the participant and, where applicable, probes for further information were made. They were then escorted to the laboratory where the participants' height and weight were recorded and capillary blood samples were collected.

Capillary blood sampling was administered by the lead researcher in accordance with the university's standard operating procedure. Capillary puncture was administered using commercially available lancets, and blood was collected in ethylenediaminetetraacetic acid coated microvette tubes. To preserve the quality of the blood samples, they were immediately placed in an ice-filled container. A blood cell count was subsequently obtained for each sample on a coulter counter (Yumizen H500 OT) and stored at -80^{o}c prior to analysis.

Extraction of DNA was undertaken at Loughborough University by a researcher conducting a parallel project with the extracted DNA data. DNA was extracted from capillary blood and bisulfite converted using EpiTect Fast LyseAll Bisulfite conversion kit (Qiagen) according to the manufacturer's protocol. The blood sample was lysed, proteins denatured, and the resulting pellet was re-suspended in phosphate-buffered saline and added directly into the bisulfite reaction. The DNA was sodium bisulfite treated and subjected to two cycles of denaturing at 95^{o}C for five minutes, and incubation at 60^{o}C for 20 minutes. Bisulfite converted DNA was desulfonated and purified using MinElute spin columns.

The methylation of four CpG sites within Exon 1 of the *TNF* promoter were measured using pyrosequencing on the PyroMark Q48 Autoprep System (Qiagen). The assay covered four methylation sites; +197 +202, +214 and +222 base pairs from the transcription start site of *TNF*. Bisulfite converted DNA was used as a template for polymerase chain reaction (PCR) amplification using the PyroMark PCR kit (Qiagen) and a Veriti thermocycler (Applied Biosystems). Using 10uL of the PCR product, CpG quantification was completed using PyroMark Q48 Advanced CpG Reagents (Qiagen) in accordance with the manufacturer's protocol. The percentage methylation of the four CpG sites was calculated using PyroMark Q48 Autoprep 2.4.1 Software (Qiagen, 2016).

Mean *TNF* methylation profiles were adjusted for leukocyte counts (Jones, Islam, Edgar & Kobor, 2017) Adjustment is important as individual differences in cell type

composition of leukocytes (e.g., age related reductions, Gowers et al., 2011) represents a potential confound when examining *TNF* methylation. Adjustment for cell type composition reduces the likelihood of false positives, or masking true effects (Jones et al., 2017). In the present study, leukocyte counts were not available for the first three participants recruited into the study due to insufficient blood draw, therefore their *TNF* measurements were classified as missing data.

Data Analysis

The data were explored using bivariate correlations and path analysis. Path analysis was developed by Sewell Wright (1921,1934) as a way of investigating correlation and causation of multiple variables simultaneously, particularly in genetic models. This analysis allows the simultaneous exploration of multiple variables, to determine the extent to which it is consistent with the observed data (Byrne, 2013).

In the first stage of analysis, bivariate correlations were used to identify potential predictors of TNF methylation. Predictors included participant characteristics (e.g., age, gender), health-related behaviour (diet, physical activity, tobacco and alcohol consumption) and health-related motivation. Subsequently, significant predictors of TNF methylation ($p < .10$) were used to construct path analysis models using Mplus (Version 8.0; Muthén & Muthén) software

The first step of path analysis identified whether higher quality motivational processes were directly associated with TNF methylation. If the first step revealed associations between health-related motivation and TNF methylation, the second step introduced significant health-related behaviours into the model. This analysis would therefore indicate the nature of the association between health-related motivation and TNF methylation, namely whether the effects were direct (i.e., health-related motivation is significantly associated with TNF methylation when considering healthy behaviour) or indirect (i.e., health-related motivation is not significantly associated with TNF methylation when considering healthy behaviour).

Results

Preliminary Analysis

Means, standard deviations, and bivariate correlations of the independent variables and adjusted *TNF* CpG scores are shown in Table 5.1. Prior to constructing substantive models, path analysis was employed using observed variables to explore what facets of healthy lifestyles might predict *TNF* methylation and warrant inclusion. Non-significant correlations were not included in substantive models, enabling the adoption of the most

Table 5.1

Means, Standard Deviations, and Bivariate Correlations of the Independent Variables and DNA Methylation of TNF CpG Mean Scores (Adjusted for White Blood Cell Counts)

Dependent variable	Actual range	*M*	*SD*	Pearson correlation
Age	18-74	32.28	14.01	-.233**
Socioeconomic status	1-9[1]	3.90	2.25	-.046
BMI	17.9-41.3	24.60	4.24	-.018
Gender (0 = male, 1 = female)	0-1	.66	.48	.025
Alcohol	0-4	1.40	.86	-.094
Smoking	0-4	.61	1.11	-.225**
Diet				
Total diet score	4-12	9.68	1.47	.113
Vegetables	1-3	2.78	.42	-.051
Fruit	1-3	2.31	.65	.182*
Fish	1-3	2.40	.92	.101
Fat	1-3	2.18	.47	-.026
Motivation				
Introjected	1-12	6.92	2.75	.129
Identified	4-12	9.97	2.25	.169
Integrated	2-16	11.80	3.81	.181*
Physical activity				
Occupational last 12 months	0-46.2	4.22	9.69	-.056
Occupational lifetime	0-48.2	8.27	11.76	-.115
Leisure last week	0-25.5	5.63	5.32	-.007
Leisure last year	0-39.0	8.50	8.53	.117
Leisure lifetime	0-47.3	11.32	8.03	.185*
Leisure last week METS	0-151.5	33.83	32.95	.015
Leisure last year METS	0-240.2	51.38	50.25	.107
Leisure lifetime METS	0-227.2	69.85	49.79	.191*

Note. * = p <.10; ** = p <.05

[1]1 = least derived, 9 = most deprived

parsimonious model. All statistical analysis was conducted using Mplus (Version 8.0; Muthén & Muthén) software.

Smoking. Smoking was significantly negatively associated with *TNF* methylation (β = -.23, *p* = .03), explaining 5.0% of the variance.

Alcohol use. Consumption of alcohol was not significantly associated with *TNF* methylation (β = -.09, *p* = .39).

Diet. The overall score for diet was not a significant predictor of adjusted *TNF* methylation (β = .08, *p* = .30). However, exploration of individual food components revealed that fruit consumption emerged (albeit weakly) as a positive influence (β = .18, *p* = .08), explaining 3.3% of the variance in *TNF* methylation. The remaining separate diet score variables of vegetables, fish and fat were non-significant (β = -.05, *p* = .64; β = .10, *p* = .35 and β = -.03, *p* = .81 respectively).

Physical activity. For occupational physical activity, neither lifetime or previous year were associated with *TNF* methylation (β = -.12, *p* = .28; β = -.05, *p* = .61 respectively). The values for LTPA over the previous week and the previous year had non-significant relationships with *TNF* methylation (β=.00, *p* = .95 and β = .12, *p* = .27, respectively). However, lifetime LTPA was positively associated with *TNF* methylation (β = .19, *p* = .08), explaining 3.4% of the variance. When LTPA was adjusted for METs, the pattern of associations was similar, but slightly stronger for lifetime LTPA (β = .19, *p* = .07); however, weekly and yearly LTPA scores remained non-significant (β = .02, *p* = .89; β = .11, *p* = .32, respectively). Lifetime LTPA adjusted for METs was therefore included in the path analysis.

Path Analysis

Preliminary analysis of identified and integrated motivation revealed a strong correlation between the two variables. Following the precedent set by previous work (e.g., Taylor, 2017), integrated and identified motivation were collapsed into one variable for further analysis (hereafter referred to as autonomous motivation). Path analysis was used to construct the direct and indirect effects. The degree of model fit was evaluated using a combination of fit indices; the Satorra-Bentler chi square statistic (S-Bχ2), the standardised root mean square residual (SRMR), the robust comparative fit index (CFI), and the robust root mean square error of approximation (RMSEA). The criteria for determining acceptable fit of the model are SRMR below .80, the CFI greater than .95 and RMSEA below .06.

Introjected motivation was not significantly associated with *TNF* methylation (β = .13, *p* = .23), and therefore the indirect effects were not pursued. Two models were constructed to explore the relationship between autonomous motivation, healthy behaviours and *TNF* methylation (Figure 5.1). The first model explored direct associations between autonomous motivation and *TNF* methylation. Model fit indices demonstrated acceptable model fit to the data (S-Bχ² (3) = 2.86, *p* = .09; SRMR = .00; CFI = 1.00; RMSEA = .00 [90% CI = .00, .00]; Hu & Bentler, 1999). Autonomous motivation was a positive predictor of *TNF* methylation (β =.18, *p* =.08). Next, the healthy behaviours identified as associated with *TNF* methylation in the preliminary analysis were included in the model as indirect effects. That is, autonomous motivation was hypothesized to predict lifestyle behaviours which, in turn predicted *TNF* methylation. Model fit indices demonstrated acceptable model fit to the data (S-Bχ² (3) = 1.87, *p* = .60; SRMR = .03; CFI = 1.00; RMSEA = .00 [90% CI = .00, .15]). Autonomous motivation was positively associated with fruit consumption (β = .29, *p* = .004), negatively associated with smoking (β = -.22, *p* = .03), but not lifetime LTPA (β = .10, *p* = .34). In turn, lifetime LTPA was positively associated with, and smoking negatively associated with, *TNF* methylation (β = .18, *p* = .08; β = -.23, *p* = .03 respectively). Fruit consumption did not remain significantly associated with *TNF* methylation when included in this model (β = .13, *p* = .21). Direct effects of autonomous motivation on DNA methylation did not persist when these indirect effects were included (β = .09, *p* = .43). Due to age being associated with adjusted *TNF* methylation scores (Gowers et al., 2011), the indirect effects model was repeated controlling for this relationship. However, model fit indices suggested this model was a poor fit for the data (S-Bχ² (6) = 15.85, *p* = .01; SRMR = .08; CFI = .68; RMSEA = .14 [90% CI = .06 - .22]).

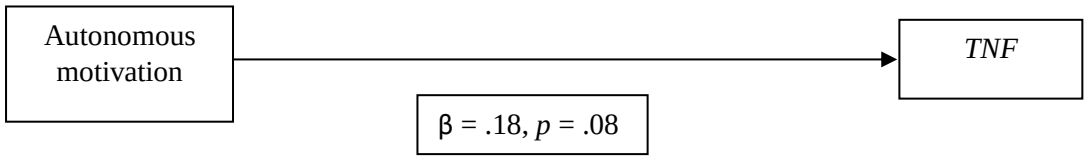
Discussion

The influence of psychological experiences in epigenetic processes have become an increasing area of interest (Jones et al., 2018), with evidence suggesting the subjective, rather than objective, experiences of the individual may be most important in explaining epigenetic modifications (Cole et al., 2007). The present research represents the first investigation of the relationship between health-related motivation and *TNF* methylation. The potential role of diet, alcohol, smoking, and physical activity were also examined in relation to epigenetic process, something which has received very little attention. The hypotheses proposed two possible roles for motivation in epigenetic processes. First, to test the direct effect of motivation on DNA methylation of exon 1 of the cytokine encoding gene *TNF*. Second, to

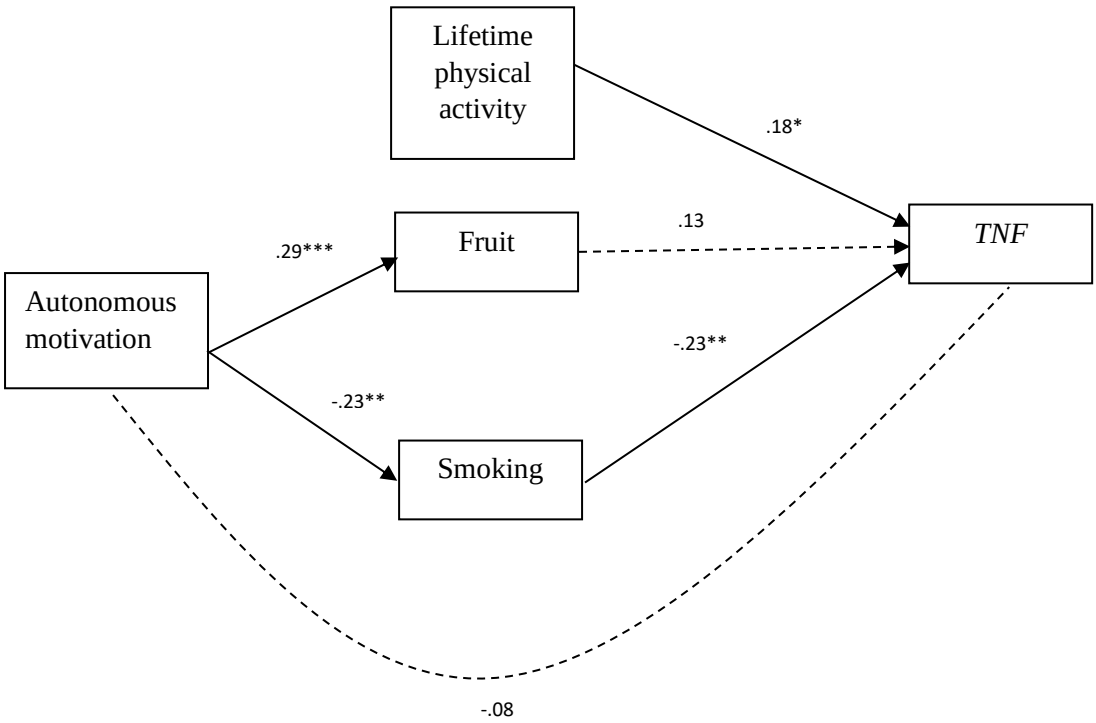
Figure 5.1.

Relationships between motivation to engage in health behaviour, healthy behaviour and TNF methylation

(a) Direct Pathway



(b) Indirect Pathway from a Multivariate Model.



* = p <.10; ** = p <.05; *** = p <.01

test if this effect persisted when including indirect effects via healthy behaviour. The first hypothesis was supported as motivational processes were associated with methylation of the *TNF* exon 1. Autonomous motivation to engage in healthy behaviour, but not introjected motivation, was positively associated with *TNF* methylation. This result supports a burgeoning area of literature that suggests the quality of motivation is an important factor in predicting downstream physiological mechanisms (Di Domenico & Ryan, 2017). Furthermore, it provides support for the implication that psychological phenomena are an important factor in epigenetic processes.

Inclusion of various healthy lifestyle behaviours rendered the direct effect of motivation non-significant. In particular, the association between autonomous motivation and *TNF* methylation was explained by participants' smoking habit. This finding may have significant implications for health if the processes are found to be causal. The only previous study that explored the relationship between smoking and *TNF* methylation found no direct relationship (Beach et al., 2017). However, this study was limited to a young adult African-American population, and therefore is not directly comparable with the present research. Previous studies have suggested higher tobacco consumption is positively associated with circulatory TNFα protein levels (e.g., Arnson, Shoenfeld & Amital, 2010), which are associated with attenuated methylation of the *TNF* gene promoter and exon 1 regions (Hermsdorff et al., 2013). Understanding the causal processes involving smoking, *TNF* methylation and circulatory TNFα could have important implications for health and disease risk, given the association of increased levels of TNFα with noncommunicable diseases including Crohn's disease and rheumatoid arthritis (Maxwell et al., 2008; Van Deventer, 1997) and noncommunicable risk factors such as obesity (Campión, et al., 2009; Milagro et al., 2011).

Within the wider literature, the association between autonomous motivation and healthy behaviour, such as smoking abstinence, physical activity and a healthy diet, is robust (Ng et al., 2012). In the present study, autonomous motivation was not associated with lifetime LTPA; however, to some extent this can be explained. The questionnaire measuring motivation towards healthy behaviour measured current motivation, and physical activity participation is strongly associated with the motivational regulation at the time of engagement (Ryan & Deci, 2017). Lifetime LTPA by design measures historical physical activity, hence, the chronological mismatch means it is not surprising to find no association between current

motivation towards healthy behaviour and historical measures of physical activity. Although lifetime LTPA was not implicated in the motivational-epigenetic processes, this was the first study to demonstrate the association of physical activity and *TNF* methylation across a wide range of ages, with previous research limited to a cohort study within an elderly population (Shaw et al., 2014). The results of the present research supported the findings of Shaw and colleagues; higher levels of long-term physical activity were associated with methylation of the *TNF* exon 1. Non-significant relationships between *TNF* methylation, and weekly and yearly levels of physical activity suggests adherence to a long-term physical activity program is required to affect *TNF* methylation. However, it would be premature to disregard acute physical activity as a potential regulatory mechanism of DNA methylation of inflammatory-related CpG sites (Robson-Ansley et al., 2014), and circulatory TNFα independent of genetic modifications (e.g., Dufaux & Order, 1989; although see Gökbel et al., 2012). Nonetheless, adherence to long-term physical activity may be beneficial in increasing methylation of the *TNF* gene and may be a potential intervention for inflammatory-related diseases. Indeed, a recent meta-analysis found physical activity combined with *TNF* inhibitor medication demonstrated reduced disease activity in patients with ankylosing spondylitis (Liang et al., 2015).

In accordance with previous evidence (e.g., Mata et al., 2009), a relationship between autonomous motivation and fruit consumption was observed, however, this did not in turn associate with *TNF* methylation. Diet and *TNF* methylation have a complex relationship (Bollati et al., 2014), however, the relatively homogeneous healthy profile of the participants in the present research may explain this finding. Authors of the DQS recommend a healthy dietary habit score above seven as an indicator of a good diet. Almost all (86 of 88) participants met this criterion, with 54 participants scoring higher than ten on the scale. Furthermore, 54 participants also had a BMI below 25. Evidence for the associations between diet and *TNF* methylation are predominately limited to populations with a higher BMI (Campión et al., 2009; Cordero et al., 2011; Milagro et al., 2011), and therefore the relatively healthy profile of the participants may explain the lack of any association in the present study.

A non-significant relationship between introjected motivation and *TNF* methylation was observed, and therefore the indirect effects of lower quality motivation through healthy behaviour were not pursued. Introjected motivation can motivate individuals towards their goals; however, as a more controlled form of motivation this effect is often short-lived and

unreliable in predicting sustained engagement with healthy behaviour (Teixeira, Carraça, Markland, Silva & Ryan, 2012). It is likely that this lower quality motivation does not lead to consistent behavioural choices which create an adaptive *TNF* methylation profile. Moreover, it highlights the difference between high and low-quality motivation. High quality motivation is theoretically and empirically associated with improved health, and the implications of the present research further extends this association to epigenetic modifications.

Limitations and Future Directions

First, the present study was largely exploratory in nature; hence, it is necessary to build on these findings with confirmatory and replication work. That said, continued exploration of associations with other genetic loci that are potentially responsive to motivation and healthy behaviour is recommended. Furthermore, exploration of genome-wide global DNA methylation, which can then be used to estimate chronological versus biological age (e.g., Horvath, 2013), may provide a novel way to extend the present research to more general indicators of health. For example, the need to examine motivational processes for their potential protective effects against epigenetic aging for individuals exposed to less supportive environments has been highlighted (Brody, Miller, Yu, Beach & Chen, 2016). Investigation of interventions designed to increase autonomous motivation as a mechanism for improving health outcomes for populations susceptible to increased TNFα levels appears an obvious line of enquiry.

Second, it is currently unknown whether the higher epigenetic plasticity characterized in early developmental periods (Calvanese, Lara, Kahn & Fraga, 2009) is particularly sensitive to the increased physical activity levels during pre-adolescence. In other words, is physical activity when young epigenetically more important than during later periods? Although this potential relationship is persuasive, there is counterevidence of a positive association between physical activity in elderly populations and methylation of the *TNF* gene (Shaw et al., 2014). As a result, the role of physical activity on *TNF* methylation throughout the lifespan is worthy of further exploration.

Third, there are methodological limitations of the present research that warrant discussion. Firstly, this study was cross-sectional in design. Although a statistical model was constructed that implied causality, the limitations of cross-sectional research prevent the determination of a cause-effect relationship between autonomous motivation, healthy behaviour and *TNF* methylation. Additionally, the population was recruited from a university campus, and were therefore relatively young, with a lower than average BMI, higher than

average physical activity, and a healthy diet. This precludes generalizing the results of this study to the general population, or to populations where increased circulatory TNFα is linked with diseases such as obesity, Crohn's disease or type one diabetes (Arroyo-Jousse et al., 2016; Van Deventer, 1997; Ye, 2008).

Conclusion

The goal of the present research was to examine associations between human motivation, healthy behaviours, and *TNF* methylation. Data were used to construct a novel exploratory model demonstrating autonomous motivation to engage in healthy behaviour was associated with tobacco consumption, which in turn was associated with *TNF* methylation. In addition, adherence to long-term physical activity was associated with *TNF* methylation. The present research extends motivational research to epigenetic processes and supports the importance of higher quality motivation for improved health.

Chapter Six

General Discussion

General Discussion

Much research has been conducted examining the reasons why people engage in goal-directed behaviour, with many motivational theories emphasizing the importance of high-quality motivation in producing not only adaptive behavioural outcomes, but improved well-being (e.g., McClelland, 1987, Dweck, 2017; Ryan & Deci, 2017). However, despite the benefits of employing physiological markers associated with improved well-being, very little research has examined motivation, behaviour and health-related biological markers concurrently. This is important as examination of biological processes provides a critical empirical link between motivational and behavioural regulation. The aim of this thesis was to address this gap in the literature. First, Chapter Two examined associations between multidimensional theories of motivation and salivary markers associated with activation of the endocrine system. Following gaps in the literature that were identified within the systematic review, Chapters Three and Four examined self-determined motivation, self-control and cortisol, a physiological marker of stress reactivity. Finally, Chapter Five investigated the motivational effect on healthy behaviour and epigenetic modifications, with the aim of exploring the potential for long-term, downstream effects of motivational processes on health. This final chapter will synthesize and integrate the key findings from each chapter and discuss the theoretical and practical implications. Furthermore, it will comment on the limitations of the present work, as well as identifying promising directions for future research.

Key findings

The present work has several novel key findings that add to both the psychology and physiology literature. First, by adopting a trans-theoretical approach to investigate endocrine-related responses, Chapter Two found consistent evidence of high-quality motivational processes attenuating the cortisol response. Previous work that had explored this idea had done so from a specific theoretical perspective (i.e., SDT, AGT or implicit theories). By systematically reviewing this literature from a trans-theoretical perspective, it validates the proposition that these theories share conceptual similarity in proposing the quality of motivation is more important that the quantity (Dweck, 2017, Roberts, 2018). As the present work identified that all these theories were associated with similar effects on the cortisol response, producing large effect sizes, this conceptual overlap can now be extended to physiological responses.

Chapter Two also called into question the assumed relationship between needs theory and hormones. Previous narrative reviews in this area have seemingly presented consistent evidence for a robust relationship between nPower, nAff and nAch (Schultheiss, 2013; Stanton & Schultheiss, 2009), and key tenet of needs theory is the assumed hormonal relationship (Schüler et al., 2018). However, a systematic review of needs theory and a variety of endocrine-related responses contradicted the assumed hormonal relationship. Firstly, in many cases, the number of significant results were outweighed by non-significant results. Moreover, where significant results were presented, research hypotheses could not be supported by consistent and coherent statistical analysis, instead relying on post-hoc analysis of single timepoints, *p*-hacking and removal of participants to obtain statistically significant results. Thus, the assumed relationships between nPower, nAff and nAch and a variety of hormonal responses including cortisol, testosterone, progesterone and norepinephrine must be questioned. The only exception to this is are the associations between nPower and nAff, and higher and lower levels of sIgA respectively, which although not always observed between studies, was present more frequently than it was absent.

In a further novel key finding, there have been over a hundred examinations of self-control ego depletion within the sequential task paradigm, however little attention has been paid to psychophysiological responses. This is an important area of investigation considering the lack of replicability of the ego-depletion effect (e.g., Carter et al, 2014), leading to calls to examine the reasons for this lack of consistency with the literature (LeBel et al., 2017). Chapters Three and Four were the first studies to explore the sequential-task paradigm while simultaneously measuring cortisol. A potential key finding was the association between an increased cortisol response and improved self-control performance evident in Chapter Three. The failure to produce a cortisol response in Chapter Five precluded further examination of this idea. Nonetheless, this represents a novel finding that is worthy of further investigation.

Furthermore, this thesis was the first to explore high-quality motivation and cortisol with participants tested individually. Previous work in this area has manipulated motivation and measured the concurrent cortisol response in groups of individuals (e.g., Hogue et al., 2017; Hogue et al., 2013; Reeve & Tseng, 2011; Yeager et al., 2016). Examination of individuals isolated from social evaluation is important as social-evaluative threat is an important component of the cortisol response, along with uncontrollability (Dickerson & Kemeny, 2004). It should be noted that the presence of the experimenter in the present work would still elicit social-evaluation, however this is likely reduced compared with the extant

literature (i.e., learning to juggle in a peer group). By testing participants individually, Chapter Three suggests high quality motivation is effective ameliorating cortisol in environments lower in social-evaluative threat. Although this idea was not supported in Chapter Four, it nonetheless represents a novel and potentially important theoretical finding.

Finally, Chapter Five was the first study to investigate motivational process and epigenetic modifications. Specifically, SDT was used as a theoretical lens to explore the methylation of DNA at the *TNF* exon 1 and promoter. This represents an important extension of the SDT literature. A key tenet of SDT is the association between higher quality motivation and improved well-being (Ng et al., 2012). By extending this concept to epigenetic processes, this thesis supports the intriguing idea that higher quality motivation is associated with downstream biological processes that may have long-term implications for health and improved human functioning. Furthermore, this thesis adds to a growing body of literature suggesting physical activity and health-related behaviours are adaptive at the genetic level. This was the first study to examine long-term physical activity and DNA methylation of the *TNF* gene across a wide-range of ages, with the only previous study restricted to an elderly population (Shaw et al., 2014). It was also the first study to find a significant association between smoking and DNA methylation of *TNF*. Increased circulatory levels of TNFα are associated with noncommunicable diseases including Crohn's disease and rheumatoid arthritis (Maxwell et al., 2008; Van Deventer, 1997) and noncommunicable risk factors such as obesity (Campión, et al., 2009; Milagro et al., 2011). As DNA methylation of the *TNF* gene is associated with reduced expression of TNFα, the present findings may have substantial implications for management of chronic health conditions associated with elevated levels of TNFα.

High-Quality Motivational Processes Attenuate Cortisol Reactivity

Chapters Two and Three supported the idea that higher quality motivation attenuates cortisol reactivity. In Chapter Two, the systematic review revealed a consistent pattern demonstrating experimental manipulations of motivation produced an adaptive cortisol response. Across self-determination theory (Ryan & Deci, 2017), achievement goal theory (AGT; Nicholls, 1984) and implicit theories (Dweck, 2016), the respective high-quality theoretical constructs of autonomous motivation, task-oriented goal environments and incremental beliefs yielded attenuated cortisol responses when compared with lower quality motivation foundations (Hogue et al., 2013; Hogue et al., 2017; Reeve & Tseng, 2011; Yeager et al., 2016). Moreover, the findings were supported by a pre-registered double-blind

experiment with excellent methodological rigor (Yeager et al., 2016, study 2). The value of high quality motivation was also supported by satisfaction of basic psychological needs – an essential ingredient for the integration of higher quality autonomous motivation – also producing an attenuated cortisol response (Quested et al., 2011). Furthermore, there was also evidence of higher quality motivation producing adaptive stress profiles with other markers of stress reactivity, with evidence of incremental beliefs and autonomy support attenuating levels of DHEA (Yeager et al., 2016, study 2), and sAA (Sieber et al., 2016) respectively.

The evidence provided by Chapter Two reinforcing the value of high-quality motivation attenuating cortisol reactivity received further support in this thesis. Chapter Three repeated the pattern evident in Chapter Two, with low-quality controlled motivation leading to a higher, and high-quality autonomous motivation leading to a lower, cortisol response. However, the evidence supporting the adaptive benefits of high-quality motivation was tempered by the results in Chapter Four. Although a significant difference was observed between experimental conditions, it was driven by a linear decline in cortisol in the neutral condition, with non-significant differences between autonomous and controlled motivational conditions. Given the weight of evidence provided by Chapters Two and Three, it is reasonable to conclude that Chapter Four was a failed attempt to replicate what appears to be a reliable effect.

Indeed, failed replications are deemed a normal pathway, particularly in social sciences, and fluctuations between time, situations and individuals are to be expected (Iso-Aloha, 2017). Recent meta-analytic work examining the replication crisis in psychology highlighted the difficulty of replicating observed effects (Stanley, Carter & Doucouliagos, 2018). The meta-analysis highlighted three key dimensions likely to affect successful replication – statistical power, selective reporting bias and between study heterogeneity. Regarding statistical power, the method for calculating the sample size for Chapter Four was based on the effect sizes observed between conditions for cortisol ($\eta^2_p = .14$) and self-control ($\eta^2_p = .11$) in Chapter Three. Even using the more conservative estimate, post hoc power analysis revealed that on the basis of $\eta^2_p = .11$, a total sample size of 70, with three experimental groups, three repeated measures and $\alpha = .05$ yielded sufficient power of 0.89. Based on large effects observed in similar studies (Hogue et al., 2013; Hogue et al., 2017; Reeve & Tseng, 2011; Yeager et al., 2016) there is little reason to believe the size of the effect used to

estimate the required sample size in Chapter Four was overestimated, and therefore insufficient power should not be a threat to the present results. Selective reporting can also be discounted as a threat to validity, due to the transparency of the analysis employed in Chapter Three and Four. Moreover, in wider psychological research, there is very little statistical evidence that questionable research practices or analysis are significant threats to replicability (Stanley et al., 2018). Finally, several changes were made to the methods of Chapter Four, rendering the replication as conceptual rather than direct (LeBel et al., 2017). Thus, the most likely threat to the failure in replicating the results of Chapter Four was increased heterogeneity. This conclusion is supported by Stanley and colleagues' meta-analysis, which also concluded heterogeneity is the likeliest threat to successful replication. Heterogeneity of effect sizes is a relative measure of between study variance that cannot be explained by the differences in the standard errors associated with effect sizes (Higgins & Thompson, 2002). Hence, the differences are most commonly attributable to differences in experimental and statistical methods, population differences and measures. Over a sample of over 12,000 effect sizes, it was found that 74% ($I^2 = 0.74$) of the variation among reported effect sizes was attributable to heterogeneity (Stanley et al., 2018). Indeed, a high-profile pre-registered replication that made significant attempts to reduce heterogeneity (Hagger et al., 2016), nonetheless reported significant amounts of heterogeneity ($I^2 = 0.36$). As Chapter Four amended several aspects of the method, including the operationalization of the independent variable, it is likely this increased heterogeneity towards the higher end of these estimates. At the average level of 74% it is unlikely replication would find an effect close to that previously reported (Stanley et al., 2018). In conclusion, the failed replication observed in Chapter Four is a limitation of this thesis. However, due to heterogeneity of effect sizes observed when attempting replication, the likelihood of successful replication should not be overestimated, nor should the significance overinterpreted.

Collectively, findings of this thesis support a key tenet of motivational theories emphasizing the quality of motivation. Self-determination theory posits that engagement with behaviour that is underpinned by greater autonomous motivation is associated with enhanced health and well-being (Ng et al., 2012; Ryan & Deci, 2017). Moreover, the benefits of task-oriented environments and incremental beliefs are also associated with improved well-being (Reinboth & Duda, 2006; Yeager et al., 2014). Activation of the endocrine system in response to a perceived threat is an adaptive process that helps the individual mobilize sufficient resources to respond to the situation (Selye, 1950). However, frequent activation of

the endocrine system is associated with suppression of immune functioning, increasing susceptibility to disease (viz. allostatic load, McEwen, 1998). Chapters Three and Four offer support for the idea that high quality motivation is directly associated with adaptive endocrine functioning via attenuating the cortisol response. By demonstrating motivational processes can moderate acute stress reactions, this thesis provides support for the proposition that motivational processes may have important health-related implications.

Influence of Motivation on Self-Control Performance

Chapters Three and Four revealed mixed findings for the effects of motivational processes on self-control performance. Evidence suggests autonomous motivation should improve, and controlled motivation should diminish, performance on the dependent measure of self-control within the sequential-task paradigm (e.g., Muraven et al., 2008). Second, although autonomous motivation should improve performance within the sequential-task paradigm, exposure to further self-control tasks should see this motivational effect dissipate (Graham et al., 2014; Vohs et al., 2012).

Chapter Four supported these propositions; participants in the autonomy-supportive condition performed significantly better than those in the controlled regulation condition on the wall sit task. Furthermore, supporting the idea that motivational effects of self-control performance do not persist over multiple self-control trials, there was no difference in performance between conditions on the final self-control task. However, support for these propositions were not equivocal due to contradictory evidence presented in Chapter Three. First, within the sequential-task paradigm, participants recorded significantly better wall sit times in the controlled regulation condition than the autonomy-supportive condition. Furthermore, this significant difference persisted to the final wall sit task.

There are several plausible explanations for the inconsistency of these results. First, and perhaps most obvious, is the debate surrounding the size of the ego depletion effect that is reported within the self-control literature. Initial estimates via meta-analysis revealed a consistent, moderate effect for ego depletion (Hagger, et al., 2010). However, more recent meta-analysis has suggested the ego depletion effect is indistinguishable from zero (Carter et al., 2015), and a recent pre-registered replication across 23 laboratories including over 2000 participants failed to find

evidence for the ego depletion effect (Hagger et al., 2016). It is important to note that, at least for published studies, the effect of autonomous motivation effect on self-control performance appears a robust one (Hagger et al., 2010). However, it may be that the ego depletion effect is not as large as was previously suggested, and failures to consistently replicate significant results using a variety of procedures may be a symptom of this (Baumeister et al., 2018).

This important point regarding the size of the ego depletion effect notwithstanding, there are reasons to believe the results observed in Chapter Four may be more reliable than those observed in Chapter Three, as several limitations that were particularly salient for the self-control tasks were addressed. First, methodological refinements were implemented, including improved control for physical activity levels, the addition of a control group, and standardization of the instructional elements to reduce experimenter interaction. Second, in Chapter Four, the second wall sit task was replaced by a similar physical task, the plank. Exposing participants to identical self-control tasks may mask ego depletion effects by the introduction of confounding variables (Chatzisarantis & Hagger, 2015). Altering the second dependent task addresses the aforementioned criticism by Chatzisarantis and Hagger, although it should be noted that there is not universal agreement among self-control theorists on this point (Lange, 2015). Third, the manipulation check measuring the successful internalization of the first self-control task was moved from the end of the experiment in Chapter Three, to directly after the experimental manipulation in Chapter Four. This resulted in a successful manipulation check, with autonomy-supported participants reporting greater interest/enjoyment and value/usefulness. In summary, due to the methodological improvements that were implemented in Chapter Four, the results can be viewed with greater confidence than the results presented in Chapter Three.

Self-Control and Cortisol

An important gap within the self-control literature that the present work sought to address was the lack of empirical research investigating self-control and cortisol. There is only limited work examining self-control and stress, with no previous studies examining cortisol within the sequential-task paradigm. The extant literature suggests, largely based on theoretical propositions, that stress is deleterious for self-control performance (Baumeister & Vohs, 2016; Muraven & Baumeister, 2000). There are two important distinctions that delineate the methodological approach adopted by this thesis compared with previous investigations into self-control and stress. First, the extant literature has mainly focused on chronic, naturalistic stressors such as examination periods (Oaten & Cheng, 2005),

occupational stress (Prem, Kubicek, Diestel & Korunka, 2016), and general life stress (Duckworth et al., 2013; Hamilton et al., 2014; Klein et al., 2017). This line of research contrasts with Chapters Three and Four, which experimentally induced an acute stress response in a controlled experimental environment. This distinction between chronic and acute stress is important. In response to a laboratory stressor, acute stress is characterized by a spike in HPA activity that returns to normal levels within a few hours (Dickerson & Kemeny, 2004). Chronic stress behaves somewhat differently, and varies according to several factors, most notably stressor type and time of onset (Miller et al., 2007). Exposure to chronic stressors typically leads to a spike in cortisol at the onset of the stressor, which subsequently takes one of two pathways. For situations where the stressor is no longer present (e.g., PTSD), time since onset is negatively associated with daily cortisol volume (Miller et al., 2007). For stressors that remain present (e.g., unemployment), cortisol profiles typically remain elevated until the stressor is removed (Miller et al., 2007). This emphasizes the importance of understanding the nature of the stressor and its potential downstream physiological effects before any broad implications can be drawn regarding self-control performance. At present, the self-control literature has demonstrated little attempt to delineate the type of stressor, how they might differ in their effects on self-control, and whether cortisol or other physiological processes might be a mechanism in these relationships (e.g., Baumeister & Vohs, 2016a). In Chapter Three, it was speculated that the cortisol response in the controlled condition may have facilitated self-control performance. Such an effect would contradict the self-control literature that proposes stress has a deleterious effect on self-control (Baumeister & Vohs, 2016a). However, it is plausible that chronic and acute stress affect self-control performance differently. This thesis presents the idea that the response to acute stress under laboratory conditions may facilitate short-term improvements in self-control performance. Acute stress is an adaptive response designed to mobilize physiological resources to assist an individual to meet environmental demands. Increased cortisol is associated with improved physical performance, particularly on strength tasks (e.g., Crewther et al., 2011). Moreover, moderate increases in cortisol are associated with improved cognitive performance (Lupien et al., 2007). The proposition that acute stress facilitates improved performance on self-control tasks, but may have deleterious long-term consequences, has intuitive appeal. A heightened sense of arousal coinciding

with energizing short-term self-control performance would clearly have adaptive advantages. However, if frequent exertion of self-control begets a stress response, this would have deleterious consequences for longer-term health (McEwen, 1998; Miller et al., 2015). Considering the wider issues of replication within the self-control literature, investigations of acute stress effects on self-control task performance may improve understanding of self-control processes and may also help to explain some of the variability in ego depletion effects.

Finally, previous research that has examined stress and self-control has not made a clear distinction between self-control and self-regulation (e.g., Oaten & Cheng, 2005). Although self-regulation and self-control are frequently used interchangeably, self-control is the mental process of overriding impulses or altering thoughts, emotions or behaviour, and often refers to trait processes (Baumeister & Vohs, 2016; Inzlicht & Schmeichel, 2012). However, self-regulation is a process that monitors goal-directed behaviour, makes necessary adjustments and acknowledge the importance of stress and coping (Carver & Vargas, 2011). According to these models, self-regulation monitors and organizes the motivational processes towards goal directed behaviour. When a threat to goal achievement occurs, this activates the potential for a stress response, subject to the individuals coping resources (Carver & Vargas, 2011; Lazarus & Folkman, 1984). For example, in Oaten and Cheng's study, during a university examination period, students reported increased smoking and caffeine consumption, and decreased healthy eating and physical activity. Using this example to illustrate the present point, when goal achievement is threatened (e.g., a successful examination period) and insufficient coping resources are available, this will likely lead to a chronic stress response that will likely not be alleviated until the examination period is complete. As it is likely that behaviour will return to normal once the stressor is alleviated, this better aligns with theoretical models of self-regulation than self-control. This point further illustrates the differences between the extant self-control literature and the present thesis. Although self-regulation and self-control share a close conceptual and theoretical framework, they are nonetheless distinct. By employing the sequential-task paradigm in Chapters Three and Four, this thesis explicitly tested self-control performance. The use of self-control and self-regulation interchangeably without consideration of the distinct processes and the potential effects can only serve to further muddy the waters and should be addressed to avoid further vagary in the literature.

Associations Between Motivation, Healthy Behaviour and DNA Methylation of *TNF* Gene

The final study in this thesis investigated the associations between motivational processes that underpin healthy behaviour and epigenetic modifications. The two-stage analysis revealed that autonomous motivation was positively associated with the DNA methylation of the *TNF* gene. Moreover, introjected motivation was not found to be significantly associated with *TNF* methylation. This supported the idea that higher, but not lower, quality motivation was associated with improved epigenetic profiles that were in turn associated with enhanced health. The second stage of the analysis used path analysis to examine whether this relationship persisted with the inclusion of healthy behaviour as a mediator. The model revealed that autonomous motivation was associated with improved dietary behaviour and reduced smoking. In turn, increased physical activity and reduced smoking was associated with *TNF* methylation; however, the direct effect of autonomous motivation on *TNF* methylation did not persist when considering healthy behaviour. It should be noted that the effect sizes between these relationships were small, however small effects are to be expected when examining epigenetic mechanisms (Jones et al., 2018). These findings imply that higher quality autonomous motivation may play an important role in energizing healthy behaviour, that in turn is associated with epigenetic modifications.

Existing research has supported the role of self-regulation (Miller et al., 2015), perceptions of social connectedness (Cole at al., 2007) and stress (Lam et al., 2013) as important psychological processes implicated in epigenetic modifications. The present findings support the idea that motivational processes play an important role in epigenetics. However, these effects are only as an initiator of behaviour, as direct effects were not observed. Chapter Five was the first study to examine self-determined motivational regulation as a potential epigenetic mechanism. The importance of integrating psychological theories with biological and physiological processes to refine and validate key theoretical tenets has been emphasized (Ryan & Deci, 2017). Therefore, the present thesis holds substantive theoretical implications. An important principle of self-determination theory (SDT) is that higher quality autonomous motivation is implicated with improved well-being (e.g., Ng et al., 2012). The present thesis illuminates the role played by high-quality autonomous motivation

in energising healthy behaviour and refines understanding of the epigenetic mechanisms that are implicated with improved health outcomes. The present work suggests that although motivation influences epigenetic processes related to the *TNF* gene, it does so indirectly via the energisation of healthy behaviour. This is distinct from the proposition that motivational processes are directly associated with biological or physiological processes, as was highlighted in Chapters Two, Three and Four. It is also important to note that the conclusions of the present research should not preclude the possibility of independent motivational effects affecting other loci-specific or global epigenetic processes. Furthermore, the implication that high-quality motivational processes are important in energizing healthy behaviour, which in turn is associated with the methylation of *TNF* gene, may have important implications for noncommunicable diseases. DNA methylation of the *TNF* gene is associated with several inflammatory-related non-communicable conditions including Crohn's disease and rheumatoid arthritis (Maxwell et al., 2008; Van Deventer, 1997). Furthermore, methylation of the *TNF* gene is also a limiting risk factor for conditions associated with poorer health outcomes such as obesity (e.g., Campión, et al., 2009). Therefore, the idea that motivational processes, either directly or indirectly, are associated with methylation of the *TNF* gene may have substantive practical implications in developing interventions to manage TNFα-related health conditions. Indeed, interventions have demonstrated promising results using physical activity to assist management of diseases associated with increased levels of TNFα (Liang et al., 2015). This thesis therefore adds further weight to the importance of healthy behaviour in improving noncommunicable health outcomes by potentially modifying gene transcription via epigenetic processes.

Future Directions

A goal of the present thesis was elucidating the use of physiological markers of health in order to support key theoretical tenets of motivational processes. Chapter Two found supporting evidence for a variety of motivational processes that were implicated with activation of the endocrine system. For example, individuals motivated by a greater nAff benefited from increased levels of sIgA, a marker of immune functioning; conversely, individuals motivated by nPower experienced suppressed immune functioning (Jemmott et al., 1980; McClelland & Kirshnit, 1988; McClelland et al., 1985). Furthermore, several motivational processes that are conceptualized as high-quality were associated with a dampened cortisol response, with an elevated cortisol response observed in conditions emphasizing low-quality motivation (Hogue et al., 2017; Hogue et al., 2013; Reeve & Tseng,

2011; Yeager et al., 2016). These associations support the broad theoretical tenets of the respective motivational theories. Future work would benefit from investigating physiological processes as a method of validating and refining theoretical positions. For example, a physiological marker of social support and relatedness that remains unexplored from a motivational perspective is the peptide hormone oxytocin. A large volume of literature has accumulated supporting the positive relationship between oxytocin and increased relatedness and social support (Taylor, 2007). Oxytocin is one of the first hormones released in response to stress and is implicated in the down-regulation of HPA and SNS activity (Heinrichs, Baumgartner, Kirschbaum & Ehlert, 2003), and increased seeking of pro-social behaviour (Taylor, Dickerson & Klein, 2002). The study of oxytocin is one possible method of investigating interactive physiological processes as a way of refining theoretical motivational tenets. For example, the cortisol response associated with motivated performance during laboratory stressors comprises of uncontrollability and social-evaluative threat. These variables two have a separate, additive effect on the cortisol response in a stressful situation (Dickerson & Kemeny, 2004). The evidence presented in this thesis demonstrates higher quality motivation leads to an attenuated cortisol response, and conversely lower quality motivation leads to an increased cortisol response; however, the psychophysiological mechanisms driving this effect are unclear. An interesting pathway to explore would be the effect of autonomous and controlled motivation on cortisol while simultaneously measuring oxytocin. If the provision of autonomy support attenuates the cortisol response without stimulating oxytocin, this would support a direct effect of autonomous motivation reducing cortisol via decreased uncontrollability. However, if autonomy support reduced cortisol, and this in turn coincided with an increase in oxytocin, this would suggest that the effect was driven by lowering social-evaluative threat (e.g., Taylor et al., 2006). The relationship between autonomy support and behaviour that is more autonomously regulated is assumed to be mediated by the satisfaction of the basic needs of autonomy, competence and relatedness (Ng et al., 2012; Ryan & Deci, 2017). Therefore, within SDT, there already exists a plausible mechanistic explanation for the latter of these theoretical positions. However, there is less emphasis placed on the role of social support within the theoretical boundaries of AGT and implicit theories. If increased oxytocin was found to be driving cortisol suppression observed in the higher quality

motivational environments, this could indicate that the lowering of social evaluative threat requires greater emphasis within these multidimensional motivational approaches.

An as-yet unexplored area for multidimensional motivational constructs is cardiovascular reactivity. A plethora of research has accumulated linking unidimensional motivational constructs with cardiovascular reactivity (Wright & Gendolla, 2012). In contrast to multidimensional constructs, unidimensional motivational processes are concerned with the direction and energization of behaviour (Elliot, 2006). A prominent unidimensional motivational theory, motivational intensity theory, suggests that effort should increase proportionately to match task difficulty; (1) to the degree that it is needed; (2) when expenditure yields a return; and, (3) as long as the task is achievable (Brehm & Self, 1989). The intensity of effort is associated with several markers of cardiovascular reactivity, most notably pre-ejection period, systolic blood pressure, and to a lesser extent heart rate (Gendolla, Wright & Richter, 2012). This idea presents several testable hypotheses that may help to facilitate trans-theoretical research. For example, task difficulty (i.e., the quantity of motivation required) has been manipulated to index cardiovascular reactivity and effort, but not task complexity. Higher quality motivation better predicts performance on more complex tasks (Cerasoli et al., 2012), in addition to the adaptive stress and self-control benefits that have been highlighted in this thesis.

Cardiovascular reactivity is a particularly useful marker in the context of the present investigation as several markers of cardiovascular reactivity have been indexed against theoretical constructs explored in this thesis. For example, heart rate variability is associated with self-regulation (Segerstrom, Hardy, Evans & Winters, 2012). Furthermore, vagal tone, a system underlying heart rate variability, is suggested as a marker of self-control (Laborde, Mosley & Thayer, 2017). Physiological markers of cardiovascular reactivity are also associated with activation of the SNS. For example, heart rate variability is associated with threat and challenge perceptions that may initiate a stress response (Thayer, Åhs, Fredrikson, Sollers & Wager, 2012). Most frequently used cardiovascular markers of stress reactivity include heart rate, systolic blood pressure and diastolic blood pressure (Krantz & Falconer, 1997). Therefore, there is evidence that cardiovascular reactivity is associated with self-regulation and motivational processes, and based on the evidence in this thesis, these psychological processes potentially modify the stress response.

These associated areas of research would benefit from an integrated research programme designed to illuminate the relationships between stress, self-regulation and

theories of motivation. First, it would further validate the present thesis by demonstrating the attenuating effect of high-quality motivation extends to other stress-related markers. Second, physiological measures of effort during the sequential-task paradigm are absent. By introducing tasks requiring self-control into unidimensional designs, effort can be indexed using pre-ejection period and systolic blood pressure, while heart-rate variability – an assumed marker of self-regulation – is monitored. Third, the proposition that higher quality motivation facilitates improved self-control performance and decreased stress response can be further elucidated by indexing psychological processes to cardiovascular markers. For example, whether high-quality motivation facilitates improved self-control performance by increased self-regulation (heart-rate variability), increased self-control (vagal tone) or increased effort (pre-ejection period). This line of research presents an exciting opportunity to extend the work of the present thesis to further explore physiological markers of health and human functioning

Limitations

The use of physiological and epigenetic markers is a significant strength of this thesis, however the use of only two markers – DNA methylation of the *TNF* gene, and cortisol – precludes the generalization of these findings. In the present study, the *TNF* gene was selected as it is associated with a reduced risk of noncommunicable diseases via reduced circulatory levels of TNFα (Hermsdoff et al., 2013), and is responsive to healthy behaviour (Shaw et al., 2014). However, there are likely numerous pathways from which one could explore this idea via DNA methylation. Firstly, this could be achieved by investigating loci-specific sites that are associated with the pathogenesis of disease and reduced health-related risks via psychological processes and behavioural regulation. Furthermore, wider exploration of the genome by measuring global DNA methylation would extend the present findings to more general markers of health via the estimation of biological age (Horvath, 2013).

The self-control tasks used on Chapters Three and Four, and in particular the physical tasks as measures of self-control also warrants consideration as a potential limitation. The wall sit has been employed as the dependent measure of ego depletion in previous research (e.g., Boat & Taylor, 2017). However, neither the wall sit or the plank are widely used dependent measures of ego depletion. Recent discussion into the failure of a high-profile multi-lab replication measuring the ego depletion effect

(Hagger et al., 2016) emphasized the importance of ensuring measures of self-control are sufficiently meaningful to the participants (Baumeister & Vohs, 2016a). It may therefore be argued that the dependent measures of ego depletion used in this thesis may not have been sufficiently meaningful. This threat to the validity of the ego depletion tasks is mitigated somewhat by the depleting task, the modified Stroop, being a well-validated depletion task (Hagger et al., 2010). Furthermore, the manipulation check employed in Chapter Four revealed the successful internalization of the modified Stroop in the autonomy-supportive condition, which was followed by improved wall sit performance compared with the controlled regulation condition. This point notwithstanding, the limitation of less-frequently used measures of self-control performance represents part of the wider debate within self-control research regarding the specific component processes exhibited by ego depletion tasks (Sripada, Kessler & Jonides, 2016).

The relative homogeneity of the sample population used in this thesis, particularly in Chapters Three and Four should also be acknowledged. A central theme of this thesis investigated the potential implications of motivation and self-regulation processes on health. The population used for Chapters Three and Four consisted mainly of relatively healthy, young university students. Furthermore, in Chapter Five, every effort was made to recruit a more diverse sample, particularly with regards to age; however, the age profile was nonetheless negatively skewed. Furthermore, other indicators of health and well-being were also above that one would expect to observe in a random sample of the population, and such a profile limits the generalisability of the present work. However, it is also worth noting that the healthier population employed in Chapter Five may have yielded some important findings that may not have been detected in a less-healthy population. For example, lifetime physical activity was positively related to DNA methylation of *TNF*. Mean physical activity scores that were higher than may have been observed in a random population may have teased out important findings that may have gone undetected in a less physically active population. Therefore, although the homogenous sample used in the present empirical work limits the generalisability of the findings, it may also have been useful in revealing the importance of a healthy lifestyle.

The ecological validity of the studies examining cortisol is a further limitation when seeking to generalize the results. In Chapters Three and Four, experimental studies were conducted to measure the acute stress response to a laboratory stressor. Furthermore, many studies included in the systematic review in Chapter Two measuring cortisol and

multidimensional motivation did so under controlled conditions. This limits the generalisability of these findings to situations where environments and behaviour can be modified to incorporate aspects of high-quality motivation to suppress cortisol. A large volume of literature has accrued demonstrating the benefits of high-quality motivation in applied areas of organizational, educational, sport and exercise psychology (Deci & Ryan, 2014; Elliot, Dweck & Yeager, 2018; Roberts & Treasure, 2018; Ryan, 2012; Shah & Gardner, 2008). Indeed, the systematic review in Chapter Two offered some support for incremental beliefs attenuating cortisol and DHEA responses in applied education (Yeager et al., 2016). Therefore, it appears prudent to pursue applied areas of research as potential avenues to extend the research presented in this thesis.

Finally, where the value of high-quality motivation in adapting physiological responses will be limited, is in dealing with chronic stressors. For example, if an individual is experiencing long-term unemployment, or is a caregiver for a disabled relative – in essence, environments with a high degree of uncontrollability - it is unlikely multidimensional motivational interventions would be suitable. However, for chronic stress such as PTSD, where a brief event leads to extended periods of threat (Miller et al., 2007), there may be some value in interventions incorporating higher quality motivation as a mechanism for reducing stress-related symptoms. For example, a recent exercise-based intervention in a population suffering from PTSD found yoga to be an effective intervention to reduce self-report symptoms (Mitchell et al., 2014). Moreover, follow-up research found that the yoga intervention group also reported reduced externally regulated motivation (Martin, Dick, Scioli-Salter & Mitchell, 2015). Thus, examination of the direct and indirect pathways between improved motivational regulation, physical activity and improved physiological stress profiles may prove to be a fruitful avenue future research.

Conclusions

This thesis supports the proposition that high-quality motivation is associated with epigenetic modifications and adaptive physiological functioning related to health. Chapter Five was the first study to explore motivational processes and epigenetic modifications. Supporting the idea that high quality motivation is an important mechanism for improved health and well-being, more autonomous reasons for engaging in healthy behaviour were associated with increased levels of healthy behaviour. In turn, increased healthy behaviour was associated with the methylation

of *TNF* gene, which is associated with improved non-communicable disease outcomes and is implicated with improved health. Chapter Two adopted a trans-theoretical perspective, compiling a systematic review of multidimensional motivational processes and activation of the endocrine system. Across theoretical boundaries, there was strong, consistent, experimental evidence for the benefits of high-quality motivation in attenuating the cortisol response in social-evaluative situations and environments. Chapters Three and Four addressed limitations identified in Chapter Two by concurrently examining self-determined motivation, self-control performance, and cortisol reactivity. Chapter Three supported the findings of Chapter Two; high-quality autonomous motivation attenuated the cortisol response, and in contrast, controlled motivation led to an increased cortisol response; however, this finding was not replicated in Chapter Four. Finally, there were contradicting findings when examining the effect of autonomous and controlled motivation on self-control performance, with improved performance observed in autonomy supportive and controlled regulation conditions in Chapters Five and Four respectively. It was speculated that increased cortisol levels may have confounded self-control performance, however evidence was equivocal. This thesis therefore addresses some pertinent gaps in the extant literature and offers some exciting avenues for future research in epigenetics and endocrinology.

Footnotes

[1] Partial-eta squared of .11 was based on a repeated measures ANOVA, with the first and second wall sits as the repeated measures factor, condition and the between subject, and PA<12 as a covariate (F (1,36) = 4.40, p = .043, η_p^2 = .11).

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Appendices

Appendix One

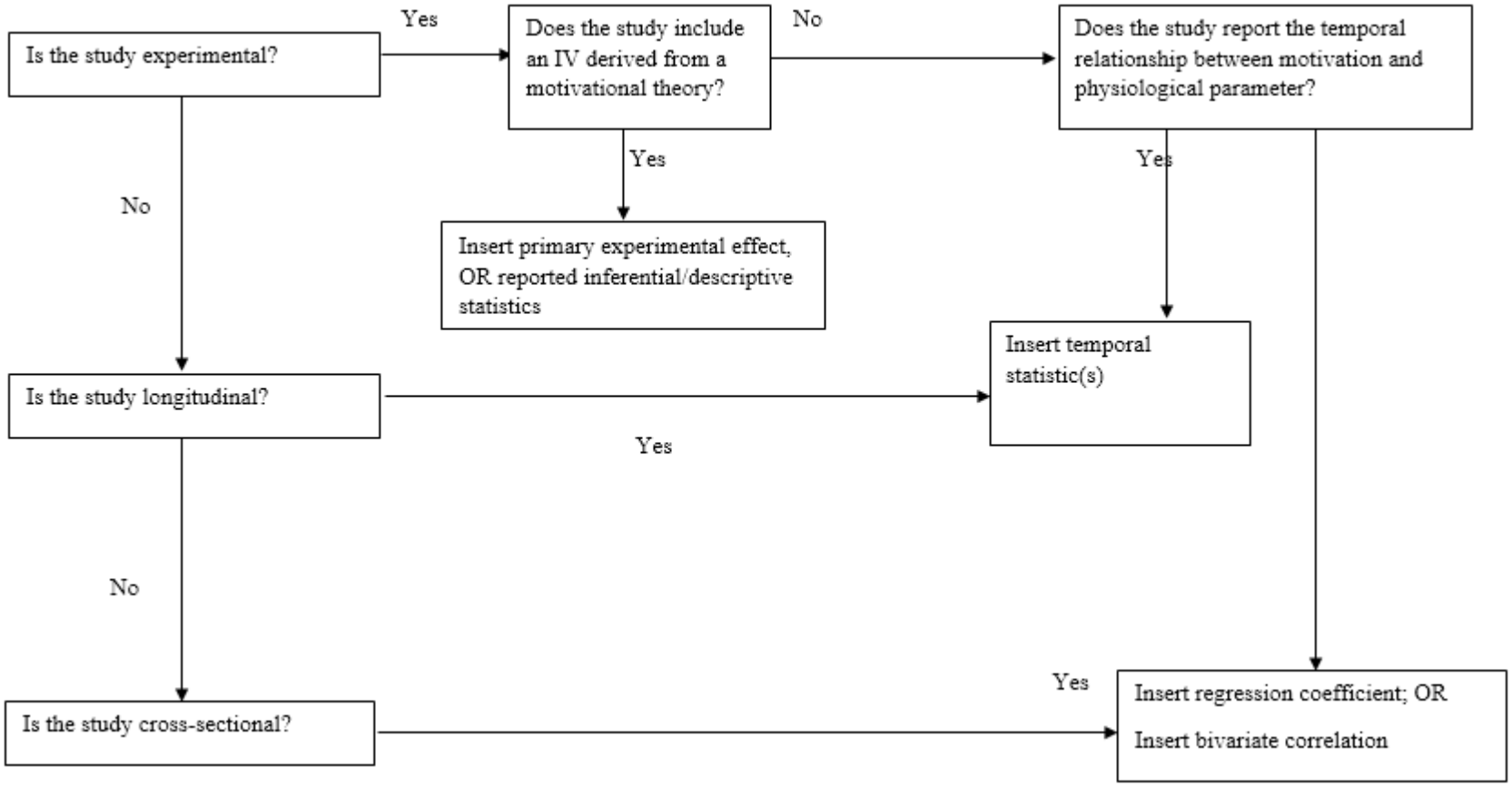
Search Terms for the Systematic Review (Study One)

Search ((((((((((((((((*endocrin*[Title/Abstract]) OR cortisol[Title/Abstract]) OR testosterone[Title/Abstract]) OR immunoglobulin*[Title/Abstract]) OR "IgA"[Title/Abstract]) OR "IgG"[Title/Abstract]) OR "IgM"[Title/Abstract]) OR ((hormon*[Title/Abstract]) NOT ((therapy[Title/Abstract] OR treatment)[Title/Abstract])))) AND Motiv*[Title/Abstract])) OR ((((((((((*endocrin*[Title/Abstract]) OR cortisol[Title/Abstract]) OR testosterone[Title/Abstract]) OR immunoglobulin*[Title/Abstract]) OR "IgA"[Title/Abstract]) OR "IgG"[Title/Abstract]) OR "IgM"[Title/Abstract]) OR ((hormon*[Title/Abstract]) NOT ((therapy[Title/Abstract] OR treatment)[Title/Abstract])))) AND Achievement goal*[Title/Abstract])) OR ((((((((((*endocrin*[Title/Abstract]) OR cortisol[Title/Abstract]) OR testosterone[Title/Abstract]) OR immunoglobulin*[Title/Abstract]) OR "IgA"[Title/Abstract]) OR "IgG"[Title/Abstract]) OR "IgM"[Title/Abstract]) OR ((hormon*[Title/Abstract]) NOT ((therapy[Title/Abstract] OR treatment)[Title/Abstract])))) AND (((self-determination[Title/Abstract]) OR "SDT"[Title/Abstract]) OR (("basic need*"[Title/Abstract]) OR "basic psychological need*"[Title/Abstract])))) OR ((((((((((*endocrin*[Title/Abstract]) OR cortisol[Title/Abstract]) OR testosterone[Title/Abstract]) OR immunoglobulin*[Title/Abstract]) OR "IgA"[Title/Abstract]) OR "IgG"[Title/Abstract]) OR "IgM"[Title/Abstract]) OR ((hormon*[Title/Abstract]) NOT ((therapy[Title/Abstract] OR treatment)[Title/Abstract])))) AND (((((entity belief*[Title/Abstract]) OR entity view[Title/Abstract]) OR entity theor*[Title/Abstract])) OR ((((Incremental belief*[Title/Abstract]) OR Incremental view[Title/Abstract]) OR Incremental theor*[Title/Abstract]))))) OR ((((((((((*endocrin*[Title/Abstract]) OR cortisol[Title/Abstract]) OR testosterone[Title/Abstract]) OR immunoglobulin*[Title/Abstract]) OR "IgA"[Title/Abstract]) OR "IgG"[Title/Abstract]) OR "IgM"[Title/Abstract]) OR ((hormon*[Title/Abstract]) NOT ((therapy[Title/Abstract] OR treatment)[Title/Abstract])))) AND self-efficacy[Title/Abstract])) OR ((((((((((*endocrin*[Title/Abstract]) OR cortisol[Title/Abstract]) OR testosterone[Title/Abstract]) OR immunoglobulin*[Title/Abstract]) OR "IgA"[Title/Abstract]) OR "IgG"[Title/Abstract]) OR "IgM"[Title/Abstract]) OR ((hormon*[Title/Abstract]) NOT ((therapy[Title/Abstract] OR treatment)[Title/Abstract])))) AND ((implicit power[Title/Abstract]) OR ((n

power[Title/Abstract]) OR npower[Title/Abstract])))) NOT ((Animal[Title/Abstract] OR mammal[Title/Abstract] OR "rat"[Title/Abstract] OR "rats"[Title/Abstract] OR mice[Title/Abstract] OR fish[Title/Abstract] OR monkey[Title/Abstract] OR chicken[Title/Abstract] OR flower[Title/Abstract] OR worm[Title/Abstract]))

Appendix Two

Data Extraction Flowchart (Study One)



Appendix Three

Intrinsic Motivation Inventory (Study Two & Three)

The following items concern your experience with the tasks. Please answer all items. For each item, please indicate how true the statement is for you.

		Not at all true	A bit true	Somewhat true	Mostly true	Completely true
1	I believe that doing this activity could be of some value for me.	1	2	3	4	5
2	I believe I had some choice about doing this activity.	1	2	3	4	5
3	While I was doing this activity, I was thinking about how much I enjoyed it.	1	2	3	4	5
4	I believe that doing this activity is useful for improved concentration.	1	2	3	4	5
5	This activity was fun to do.	1	2	3	4	5
6	I think this activity is important for my improvement.	1	2	3	4	5
7	I enjoyed doing this activity very much.	1	2	3	4	5
8	I really did not have a choice about doing this activity.	1	2	3	4	5
9	I did this activity because I wanted to.	1	2	3	4	5
10	I think this is an important activity.	1	2	3	4	5
11	I felt like I was enjoying the activity while I was doing it.	1	2	3	4	5
12	I thought this was a very boring activity.	1	2	3	4	5
13	It is possible that this activity could improve my studying habits.	1	2	3	4	5

14	I felt like I had no choice but to do this activity.	1	2	3	4	5
15	I thought this was a very interesting activity.	1	2	3	4	5

		Not at all true	A bit true	Somewhat true	Mostly true	Completely true
16	I am willing to do this activity again because I think it is somewhat useful.	1	2	3	4	5
17	I would describe this activity as very enjoyable.	1	2	3	4	5
18	I felt like I had to do this activity.	1	2	3	4	5
19	I believe doing this activity could be somewhat beneficial for me.	1	2	3	4	5
20	I did this activity because I had to.	1	2	3	4	5
21	I believe doing this activity could help me do better in university.	1	2	3	4	5
22	While doing this activity, I felt like I had a choice.	1	2	3	4	5
23	I would describe this activity as very fun.	1	2	3	4	5
24	I felt like it was not my own choice to do this activity.	1	2	3	4	5
25	I would be willing to do this activity again because it has some value for me.	1	2	3	4	5

Appendix Four

Brief Mood Inventory Scale (Study Two & Three)

INSTRUCTIONS: Circle the response on the scale below that indicates how well each adjective or phrase describes your present mood

		Definitely do not feel	Do not feel	Slightly feel	Definitely feel
1	Lively	1	2	3	4
2	Happy	1	2	3	4
3	Sad	1	2	3	4
4	Tired	1	2	3	4
5	Caring	1	2	3	4
6	Content	1	2	3	4
7	Gloomy	1	2	3	4
8	Jittery	1	2	3	4
9	Drowsy	1	2	3	4
10	Grouchy	1	2	3	4
11	Peppy	1	2	3	4

12	Nervous	1	2	3	4
13	Calm	1	2	3	4
14	Loving	1	2	3	4
15	Fed Up	1	2	3	4
16	Active	1	2	3	4

Appendix Five

Borg Scale – Mental Exertion (Study Two & Three)

Borg Scale – Mental exertion

Please rate your mental **exertion** in the physical task. Please circle your answer.

0	Nothing at all
0.5	Extremely Weak (Just Noticeable)
1	Very Weak
2	Weak (Light)
3	Moderate
4	
5	Strong (Heavy)
6	
7	Very Strong
8	
9	
10	Extremely Strong (Almost Max)

Appendix Six

Borg Scale – Physical Exertion (Study Two & Three)

Borg Scale – Physical exertion

Please rate your **physical exertion** in the cognitive task. Please circle your answer.

0	Nothing at all
0.5	Extremely Weak (Just Noticeable)
1	Very Weak
2	Weak (Light)
3	Moderate
4	
5	Strong (Heavy)
6	
7	Very Strong
8	
9	
10	Extremely Strong (Almost Max)

Appendix Seven

Index of Autonomous Functioning (Study Two & Three)

Gender: Male / Female **Age:** _____ years

Below is a collection of statements about your general experiences. Please indicate how true each statement is of your experiences on the whole. Remember that there are no right or wrong answers. Please answer according to what really reflects your experience rather than what you think your experience should be.

		Not at all true	A bit true	Somewhat true	Mostly true	Completely true
1	My decisions represent my most important values and feelings	1	2	3	4	5
2	I do things in order to avoid feeling badly about myself	1	2	3	4	5
3	I often reflect on why I react the way I do	1	2	3	4	5
4	I strongly identify with the things that I do	1	2	3	4	5
5	I do a lot of things to avoid feeling ashamed	1	2	3	4	5
6	I am deeply curious when I react with fear or anxiety to events in my life	1	2	3	4	5
7	My actions are congruent with who I really am	1	2	3	4	5
8	I try to manipulate myself into doing certain things	1	2	3	4	5
9	I am interested in understanding the reasons for my actions	1	2	3	4	5
10	My whole self stands behind the important decisions I make	1	2	3	4	5
11	I believe certain things so that others will like me	1	2	3	4	5
12	I am interested in why I act the way I do	1	2	3	4	5

13	My decisions are steadily informed by things I want or care about	1	2	3	4	5
14	I often pressure myself	1	2	3	4	5
15	I like to investigate my feelings	1	2	3	4	5

Appendix Eight

Questionnaire Pack (Study Two)

The Effect of Physical and Cognitive Performance on Cortisol

Adult Participant Information Sheet

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What is the purpose of the study?

The study aims to investigate the relationship between cognitive and small physical effort, and the associated effect on hormonal and immune responses in saliva.

Who is doing this research and why?

The lead investigator is Richard Steel, a doctoral candidate at Loughborough University. The supervisor of the project is Dr Ian Taylor, a senior lecturer in the School of Sport, Exercise, and Health Sciences at Loughborough University.

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Are there any exclusion criteria?

Participants must be aged 18 or over. Children under the age of 18 cannot participate.

What will I be asked to do?

You will be asked to take part in a cognitive task of your choice, and also provide measures of physical performance, via a wall sit. You will also be asked to provide saliva samples by "drooling" into a collection tube. Finally, you will be asked to complete a couple of brief questionnaires.

Once I take part, can I change my mind?

Yes. After you have read this information and asked any questions you may have we will ask you to complete an Informed Consent Form, however if at any time, before, during or after the sessions you wish to withdraw from the study please just contact the main investigator. You can withdraw at any time, for any reason and you will not be asked to explain your reasons for withdrawing). You will be able to request that your data is withdrawn from the study up to 2 months from your participation in the study. After this time, it may not be possible for you to withdraw your data from the study as the data may have been aggregated or published.

Will I be required to attend any sessions and where will these be?

You will be required to attend one session, at the National Centre for Sport and Exercise Medicine at Loughborough University.

How long will it take?

The experiment is expected to take approximately one hour.

What personal information will be required from me?

You will not be required to provide any information of a personal nature.

Are there any risks in participating?

No.

Is there anything I need to do before the sessions?

Providing a good saliva sample requires you to abstain or document certain activities prior to taking part in the experiment. We would ask you:

1. Do not eat a major meal for at least two hours prior to the start of your session.
2. Do not eat any food or drink other than water for at least 60 minutes prior to the session.

We will also ask you to document vigorous physical activity and the presence of oral diseases or injury, and also provide information on any alcohol, caffeine, nicotine, and prescription/over-the-counter medications consumed within the prior 12 hours.

These measures are to prevent contamination of the saliva, which can affect the experimental results.

What do I get for participating?

If you are an undergraduate on the BSc psychology programme, you may receive course credit towards your research methods module(s)

Will my taking part in this study be kept confidential?

All data will be handled in line with the Data Protection Act (1998). All data will be coded and logged on a password protected computer; completed questionnaires will be stored in a locked cupboard and retained for six years. Upon agreement with the participant, saliva samples will be stored until 31/10/2019 for future research in the same theme as this project.

I have some more questions; who should I contact?

The lead investigator, Richard Steel (details above).

What will happen to the results of the study?

The results will be submitted to Loughborough University as part of a PhD thesis and published in psychology journals (no identifying details will be in any communication).

What if I am not happy with how the research was conducted?

If you are not happy with how the research was conducted, please contact Ms Jackie Green, the Secretary for the University's Ethics Approvals (Human Participants) Sub-Committee:

Ms J Green, Research Office, Hazlerigg Building, Loughborough University, Epinal Way, Loughborough, LE11 3TU. Tel: 01509 222423. Email: J.A.Green@lboro.ac.uk

The University also has a policy relating to Research Misconduct and Whistle Blowing which is available online at http://www.lboro.ac.uk/committees/ethics-approvals-human-participants/additionalinformation/codesofpractice/

Introjected and Identified Motivation as Predictors of Self-Control Depletion and Biomarkers in Saliva

INFORMED CONSENT FORM
(to be completed after Participant Information Sheet has been read)

Taking Part	**Please initial box**
The purpose and details of this study have been explained to me. I understand that this study is designed to further scientific knowledge and that all procedures have been approved by the Loughborough University Ethics Approvals (Human Participants) Sub-Committee.	☐
I have read and understood the information sheet and this consent form.	☐
I have had an opportunity to ask questions about my participation.	☐
I understand that I am under no obligation to take part in the study, have the right to withdraw from this study at any stage for any reason, and will not be required to explain my reasons for withdrawing.	☐
I agree to take part in this study.	☐

Use of Information

I understand that all the personal information I provide will be treated in strict confidence and will be kept anonymous and confidential to the researchers unless (under the statutory obligations of the agencies which the researchers are working with), it is judged that confidentiality will have to be breached for the safety of the participant or others or for audit by regulatory authorities.	☐

Bodily Samples

I agree that the saliva samples taken during this study can be stored until 31/10/2018 for future research in the same research theme as this project.	☐

_____	_____	_____
Name of participant [printed]	Signature	Date
_____	_____	_____
Researcher [printed]	Signature	Date

Appendix Nine

Experimental Manipulation (Study Two & Three)

You now have a choice of the forthcoming task. Please indicate which one you wish to participate in:

a) The Mental Distraction Game ☐

b) A Game of Accuracy ☐

c) Ignore Your Impulses ☐

d) Cognitive Response Latency Test ☐

Appendix Ten

Instructions for the Stroop Task (Study Four)

The instructions to the task

(Control condition)

You are about to be presented with some coloured words.

You are required to press the key corresponding to the COLOUR of the print ink, and ignore the text for each word presented. For example, the word "GREEN" in blue ink, you should press the blue key.

BUT...

When a word presented in RED INK, you are required to override the general instructions and press the key corresponding the presented WORD. For example, the word "GREEN" in RED INK, you should press the GREEN key.

The colours that you will see include:

Red

Green

Yellow

Blue

Press any key to start the experiment.

Appendix Eleven

International Physical Activity Questionnaire (Study Four)

INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE (IPAQ)

We are interested in finding out about the kinds of physical activities that people do as part of their everyday lives. The questions will ask you about the time you spent being physically active in the **last 7 days**. Please answer each question even if you do not consider yourself to be an active person. Please think about the activities you do at work, as part of your house and yard work, to get from place to place, and in your spare time for recreation, exercise or sport.

Think about all the **vigorous** activities that you did in the **last 7 days**. **Vigorous** physical activities refer to activities that take hard physical effort and make you breathe much harder than normal. Think *only* about those physical activities that you did for at least 10 minutes at a time.

1. During the **last 7 days**, on how many days did you do **vigorous** physical activities like heavy lifting, digging, aerobics, or fast bicycling?

_____ days per week

No vigorous physical activities ***Skip to question 3***

2. How much time did you usually spend doing **vigorous** physical activities on one of those days?

_____ hours per day

_____ minutes per day

Don't know/Not sure

Think about all the **moderate** activities that you did in the **last 7 days**. **Moderate** activities refer to activities that take moderate physical effort and make you breathe somewhat harder than normal. Think only about those physical activities that you did for at least 10 minutes at a time.

3. During the **last 7 days**, on how many days did you do **moderate** physical activities like carrying light loads, bicycling at a regular pace, or doubles tennis? Do not include walking.

_____ days per week

No moderate physical activities ***Skip to question 5***

4. How much time did you usually spend doing **moderate** physical activities on one of those days?

_____ hours per day

_____ minutes per day

Don't know/Not sure

Think about the time you spent **walking** in the **last 7 days**. This includes at work and at home, walking to travel from place to place, and any other walking that you have done solely for recreation, sport, exercise, or leisure.

5. During the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time?

_____ days per week

No walking ***Skip to question 7***

6. How much time did you usually spend **walking** on one of those days?

_____ hours per day

_____ minutes per day

Don't know/Not sure

The last question is about the time you spent **sitting** on weekdays during the **last 7 days**. Include time spent at work, at home, while doing course work and during leisure time. This may include time spent sitting at a desk, visiting friends, reading, or sitting or lying down to watch television.

7. During the **last 7 days**, how much time did you spend **sitting** on a **week day**?

_____ hours per day

_____ minutes per day

Don't know/Not sure

This is the end of the questionnaire, thank you for participating.

Appendix Twelve

Questionnaire Pack (Study Three)

Effect of Concentration and Physical Performance on Endocrine Responses

Adult Participant Information Sheet

Investigators: Richard Steel,
Loughborough University,
Leicestershire,
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Dr Ian Taylor,
Loughborough University,
Leicestershire,
LE11 3TU,
I.M.Taylor@lboro.ac.uk
01509 223274

What is the purpose of the study?

The study aims to investigate the relationship between concentration and small physical effort, and the associated effect on endocrine responses, measured via saliva.

Who is doing this research and why?

The lead investigator is Richard Steel, a doctoral candidate at Loughborough University. The supervisor of the project is Dr Ian Taylor, a senior lecturer in the School of Sport, Exercise, and Health Sciences at Loughborough University.

.

Are there any exclusion criteria?

Participants must be aged 18 or over. Children under the age of 18 cannot participate.

What will I be asked to do?

After providing informed consent, you will be given a choice of cognitive task to complete, and then provide a measure of physical performance, namely a wall sit. This will be followed by a repeat of the cognitive task and a second measure of physical performance, a plank. We will also ask you to fill out a couple of brief questionnaires during the study, and provide three saliva samples via the passive drool method at the beginning, middle and end of the study.

Once I take part, can I change my mind?

Yes. After you have read this information and asked any questions you may have we will ask you to complete an Informed Consent Form, however if at any time, before, during or after the sessions you wish to withdraw from the study please just contact the main investigator. You can withdraw at any time, for any reason and you will not be asked to explain your reasons for withdrawing. You will be able to request that your data is withdrawn from the study up to 2 months from your participation in the study. After this time, it may not be possible for you to withdraw your data from the study as the data may have been aggregated or published.

Will I be required to attend any sessions and where will these be?

You will be required to attend one session at the National Centre for Sport and Exercise Medicine at Loughborough University.

How long will it take?

The experiment is expected to take less than one hour.

What personal information will be required from me?

You will not be required to provide any information of a personal nature.

Are there any risks in participating?

No.

Is there anything I need to do before the sessions?

Providing a good saliva sample requires you to abstain or document certain activities prior to taking part in the experiment. We would ask you:

1. Do not eat a major meal for at least two hours prior to the start of your session.
2. Do not eat any food or drink other than water for at least 60 minutes prior to the session.

We will also ask you to abstain from vigorous physical activity for 24 hours prior to the study; document the presence of oral diseases or injury; and provide information on any alcohol, caffeine, nicotine, and prescription/over-the-counter medications consumed in the prior 12 hours. These measures are to prevent contamination of the saliva which can affect the experimental results.

What do I get for participating?

The study may be counted towards the research participation scheme for eligible students.

Will my taking part in this study be kept confidential?

All data will be handled in line with the Data Protection Act (1998). All data will be coded and logged on a password protected computer; completed questionnaires will be stored in a locked cupboard and retained for six years. Upon agreement with the participant, saliva samples will be stored until 31/10/2019 for future research in the same theme as this project.

I have some more questions; who should I contact?

The lead investigator, Richard Steel (details above).

What will happen to the results of the study?

The results will be submitted to Loughborough University as part of a PhD thesis and published in psychology journals (no identifying details will be in any communication).

What if I am not happy with how the research was conducted?

If you are not happy with how the research was conducted, please contact Ms Jackie Green, the Secretary for the University's Ethics Approvals (Human Participants) Sub-Committee:

Ms J Green, Research Office, Hazlerigg Building, Loughborough University, Epinal Way, Loughborough, LE11 3TU. Tel: 01509 222423. Email: J.A.Green@lboro.ac.uk

The University also has a policy relating to Research Misconduct and Whistle Blowing which is available online at http://www.lboro.ac.uk/committees/ethics-approvals-human-participants/additionalinformation/codesofpractice/

The Effect of Concentration and Physical Performance on Endocrine Responses

INFORMED CONSENT FORM

(to be completed after Participant Information Sheet has been read)

Taking Part	**Please initial box**
The purpose and details of this study have been explained to me. I understand that this study is designed to further scientific knowledge and that all procedures have been approved by the Loughborough University Ethics Approvals (Human Participants) Sub-Committee.	☐
I have read and understood the information sheet and this consent form.	☐
I have had an opportunity to ask questions about my participation.	☐
I understand that I am under no obligation to take part in the study, have the right to withdraw from this study at any stage for any reason, and will not be required to explain my reasons for withdrawing.	☐
I agree to take part in this study.	☐

Use of Information

I understand that all the personal information I provide will be treated in strict confidence and will be kept anonymous and confidential to the researchers unless (under the statutory obligations of the agencies which the researchers are working with), it is judged that confidentiality will have to be breached for the safety of the participant or others or for audit by regulatory authorities.	☐

Bodily Samples

I agree that the saliva samples taken during this study can be stored until 31/10/2019 for future research in the same research theme as this project;	☐
OR I agree that the saliva samples taken during this study can only be used for this study	☐

_____	_____	_____
Name of participant [printed]	Signature	Date
_____	_____	_____
Researcher [printed]	Signature	Date

The Effect of Concentration and Physical Performance on Endocrine Responses

Providing a good saliva sample requires you to abstain or document certain activities prior to taking part in the experiment.

	Yes	No
Have you eaten major meal in the last two hours?	☐	☐
If "yes", please provide details_____		
_____		
Have you consumed any food or drink other than water in the last 60 minutes?	☐	☐
If "yes", please provide details_____		
_____		
<u>In the last twenty-four hours, have you:</u>		
Done any vigorous physical activity?	☐	☐
If "yes", please provide details_____		
_____		
Consumed any alcohol?	☐	☐
If "yes", please provide details_____		
_____		
Consumed any nicotine?	☐	☐
If "yes", please provide details_____		
_____		
Taken any prescription medication?	☐	☐
If "yes", please provide details_____		
_____		

Appendix Thirteen

Manipulated Instructions for the Stroop Task (Study Four)

(Autonomous condition)

You are about to be presented with some coloured words.

We would like you to press the key corresponding to the COLOUR of the print ink, and ignore the text for each word presented. For example, the word “GREEN” in blue ink, the correct response is the BLUE key.

HOWEVER,

When a word presented in RED INK, we would ask you to ignore the general instruction above, and press the key corresponding the presented WORD. For example, the word “GREEN” in RED INK, the correct response is the GREEN key.

The colours that you will see include:

Red

Green

Yellow

Blue

If you are willing to participate, please begin the activity by pressing the space bar.

(Controlled condition)

You are about to be presented with some coloured words.

You must press the key corresponding to the COLOUR of the print ink, and ignore the text for each word presented. For example, the word “GREEN” in blue ink, you must press the blue key.

BUT,

When a word presented in RED INK, you must override the general instruction above, and press the key corresponding the presented WORD. For example, the word “GREEN” in RED INK, you must press the GREEN key.

The colours that you will see include:

Red

Green

Yellow

Blue

You should start the experiment by pressing the space bar.

(Control condition)

You are about to be presented with some coloured words.

You are required to press the key corresponding to the COLOUR of the print ink, and ignore the text for each word presented. For example, the word “GREEN” in blue ink, you should press the blue key.

BUT...

When a word presented in RED INK, you are required to override the general instructions and press the key corresponding the presented WORD. For example, the word “GREEN” in RED INK, you should press the GREEN key.

The colours that you will see include:

Red

Green

Yellow

Blue

Press any key to start the experiment.

Appendix Fourteen

Questionnaire Pack (Study Five)

Motivation, and Indicators of Health in Blood

Adult Participant Information Sheet

Investigators: Richard Steel,
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Dr Ian Taylor,
Loughborough University,
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Dr. Sarabjit Mastana (S.S.Mastana@lboro.ac.uk); Dr Martin Lindley (M.R.Lindley@lboro.ac.uk); Bethan Locket (b.c.l.lockett@lboro.ac.uk); David Hunter (d.j.hunter@lboro.ac.uk); Sam Wood (S.Woods-15@student.lboro.ac.uk); Jamie Smith (J.Smith-12@student.lboro.ac.uk.)

What is the purpose of the study?

The purpose of this study is to investigate if the quality of a person's motivation to engage in healthy behaviour can have negative health implications, measured via biomarkers in blood.

Who is doing this research and why?

The lead investigator is Richard Steel, a doctoral candidate at Loughborough University. The supervisor of the project is Dr Ian Taylor, a senior lecturer in the School of Sport, Exercise, and Health Sciences at Loughborough University.

.

Are there any exclusion criteria?

Participants must be aged 18 or over and be free of any bloodborne viruses. Children under the age of 18 cannot participate.

What will I be asked to do?

You will be asked to complete a couple of questionnaires about your general health, diet and exercise history, provide basic anthropomorphic measurements (height, weight, etc) and provide a small sample of blood, taken via a finger prick.

Once I take part, can I change my mind?

Yes. After you have read this information and asked any questions you may have we will ask you to complete an Informed Consent Form, however if at any time, before, during or after the sessions you wish to withdraw from the study, simply contact the main investigator. You can withdraw at any time, for any reason and you will not be asked to explain your reasons for withdrawing. You will be able to request that

your data is withdrawn from the study up to two months from your participation in the study. After this time, it may not be possible for you to withdraw your data from the study as the data may have been aggregated or published.

Will I be required to attend any sessions and where will these be?

You will be required to attend one session, at the National Centre for Sport and Exercise Medicine at Loughborough University.

How long will it take?

The study is expected to take approximately forty-five minutes.

What personal information will be required from me?

You will be asked to consent to having basic anthropomorphic measurements such as height and weight. The questionnaires will also include health-related lifestyle questions, such as drinking or smoking habits.

Are there any risks in participating?

As some of the questions are about significant life events, there is a small risk that an event which was considerably disturbing could be recalled. If this scenario occurred, you will be reminded that you can stop the study at any point in time without negative consequences. In addition, contact details for the Samaritans, chaplaincy and counselling services are provided below.

Chaplaincy: http://www.lboro.ac.uk/services/cfs/chaplains/
Counselling services: http://www.lboro.ac.uk/disabilities/counselling/
Samaritans: http://www.samaritans.org/how-we-can-help-you/contact-us

Is there anything I need to do before the sessions?

No.

What do I get for participating?

There are no incentives for participation in this study.

Will my taking part in this study be kept confidential?

All data will be handled in line with the Data Protection Act (1998). All data will be coded and logged on a password protected computer; completed questionnaires will be stored in a locked cupboard and retained for six years. Upon agreement with the participant, samples will be stored until 31/10/2019 for future research in the same theme as this project.

I have some more questions; who should I contact?

The lead investigator, Richard Steel (details above).

What will happen to the results of the study?

The results will be submitted to Loughborough University as part of a PhD thesis and published in psychology journals (no identifying details will be in any communication).

What if I am not happy with how the research was conducted?

If you are not happy with how the research was conducted, please contact Ms Jackie Green, the Secretary for the University's Ethics Approvals (Human Participants) Sub-Committee:
Ms J Green, Research Office, Hazlerigg Building, Loughborough University, Epinal Way, Loughborough, LE11 3TU. Tel: 01509 222423. Email: J.A.Green@lboro.ac.uk
The University also has a policy relating to Research Misconduct and Whistle Blowing which is available online at http://www.lboro.ac.uk/committees/ethics-approvals-human-participants/additionalinformation/codesofpractice/

INFORMED CONSENT FORM
(to be completed after Participant Information Sheet has been read)

Taking Part **Please initial box**

The purpose and details of this study have been explained to me. I understand that this study is designed to further scientific knowledge and that all procedures have been approved by the Loughborough University Ethics Approvals (Human Participants) Sub-Committee. ☐

I have read and understood the information sheet and this consent form. ☐

I have had an opportunity to ask questions about my participation. ☐

I understand that I am under no obligation to take part in the study, have the right to withdraw from this study at any stage for any reason, and will not be required to explain my reasons for withdrawing. ☐

I agree to take part in this study. ☐

Use of Information

I understand that all the personal information I provide will be treated in strict confidence and will be kept anonymous and confidential to the researchers unless (under the statutory obligations of the agencies which the researchers are working with), it is judged that confidentiality will have to be breached for the safety of the participant or others or for audit by regulatory authorities. ☐

Bodily Samples

To the best of my knowledge, I am **not** carrying any bloodborne viruses (e.g. Hepatitis, HIV). ☐

Please select only one option:

I agree that the bodily samples taken during this study can be stored until 31/10/2018 for future research in the same research theme as this project. ☐

[Or] I agree that the bodily samples taken during this study can **only be** used for this study and will be disposed of upon completion of the research [30/12/2016]. ☐

_____	_____	_____
Name of participant [printed]	Signature	Date
_____	_____	_____
Researcher [printed]	Signature	Date

	Yes	No
Have you eaten major meal in the last two hours?	☐	☐
If "yes", please provide details_____		
_____		
Have you consumed any food or drink other than water in the last 60 minutes?	☐	☐
If "yes", please provide details_____		
_____		
<u>In the last twelve hours, have you:</u>		
Done any vigorous physical activity?	☐	☐
If "yes", please provide details_____		
_____		
Consumed any alcohol?	☐	☐
If "yes", please provide details_____		
_____		
Consumed any nicotine?	☐	☐
If "yes", please provide details_____		
_____		
Taken any prescription medication?	☐	☐
If "yes", please provide details_____		
_____		

What time did you wake up this morning?_____

About You

Age_____ Postcode_____

Height_____cm Weight_____kg

Gender: Male / Female

General lifestyle questions

Do you smoke? Yes [ ] No [ ]

If Yes, do you smoke:

less than 1 cigarette/day	[ ]
1- 9 cigarettes/day	[ ]
10-19 cigarettes/day	[ ]
20-39 cigarettes/day	[ ]
More than 40 cigarettes/day	[ ]
Smoke a pipe	[ ]
Smoke cigars	[ ]

If No, have you:

Never ever smoked	[ ]
Given up smoking in the last year	[ ]
Not smoked for more than 1 year	[ ]

Alcohol

Please tick the statement which most closely describes your usual average alcohol intake. (1 unit is 1 glass wine, ½ pint beer or a single measure of spirit.)

I never drink alcohol	[ ]
I drink less than 1 unit per day	[ ]
I drink between 1 and 2 units a day	[ ]

I drink between 3 and 6 units a day [ ]

I drink between 7 and 9 units a day [ ]

I drink more than 9 units a day [ ]

Diet

Vegetables (cooked or raw) and/or vegetarian dishes

5–7 servings/week [ ]

3–4 servings/week [ ]

Less than 2 servings/week [ ]

Fruit

3 pieces/day [ ]

More than 3 pieces/week and less than 2 pieces/day [ ]

Less than 2 pieces/week [ ]

Fish

More than 200 g/week [ ]

Less than 200 g/week [ ]

No intake [ ]

Fat: bread

None [ ]

Vegetable margarine [ ]

Butter, blended spread, lard [ ]

Fat: cooking

None/olive oil [ ]

Vegetable margarine, oil [ ]

Margarine/butter/blended spread/lard [ ]

About your personality…

Below is a collection of statements about your general experiences. Please indicate how true each statement is of your experiences on the whole. Remember that there are no right or wrong answers. Please answer according to what really reflects your experience rather than what you think your experience should be.

		Not at all true	A bit true	Somewhat true	Mostly true	Completely true
1	My decisions represent my most important values and feelings	1	2	3	4	5
2	I do things in order to avoid feeling badly about myself	1	2	3	4	5
3	I often reflect on why I react the way I do	1	2	3	4	5
4	I strongly identify with the things that I do	1	2	3	4	5
5	I do a lot of things to avoid feeling ashamed	1	2	3	4	5
6	I am deeply curious when I react with fear or anxiety to events in my life	1	2	3	4	5
7	My actions are congruent with who I really am	1	2	3	4	5
8	I try to manipulate myself into doing certain things	1	2	3	4	5
9	I am interested in understanding the reasons for my actions	1	2	3	4	5
10	My whole self stands behind the important decisions I make	1	2	3	4	5
11	I believe certain things so that others will like me	1	2	3	4	5
12	I am interested in why I act the way I do	1	2	3	4	5
13	My decisions are steadily informed by things I want or care about	1	2	3	4	5
14	I often pressure myself	1	2	3	4	5
15	I like to investigate my feelings	1	2	3	4	5

WHY DO YOU ENGAGE IN HEALTHY BEHAVIOUR?

We are interested in the reasons underlying peoples' decisions to engage or not engage in healthy lifestyles. Using the scale below, please indicate to what extent each of the following items is true for you. Please note that there are no right or wrong answers and no trick questions. We simply want to know how you personally feel about being healthy. Your responses will be held in confidence and only used for our research purposes.

		Not true for me		**Sometimes true for me**		**Very true for me**
1	Being healthy is important and beneficial for my health and lifestyle	0	1	2	3	4
2	Because I would feel bad about myself if I didn't do it	0	1	2	3	4
3	I am healthy because it is consistent with my life goals	0	1	2	3	4
4	Because I'd be afraid of falling too far out of shape	0	1	2	3	4
5	Because it is personally important for me to be healthy	0	1	2	3	4
6	I consider healthy behaviour a fundamental part of who I am	0	1	2	3	4
7	Because I feel pressured to be healthy	0	1	2	3	4
8	Because I have a strong value for being healthy	0	1	2	3	4
9	I consider healthy behaviour part of my identity	0	1	2	3	4
10	I consider healthy behaviour consistent with my values	0	1	2	3	4

APPENDIX: PHYSICAL-ACTIVITY QUESTIONNAIRE AND INTERVIEWER'S INSTRUCTIONS FOR ADMINISTRATION*

Physical-activity questionnaire

Age _____ Sex _____ Date _____
Weight (kg) _____ Interviewer code _____

1. In general, about how many hours per week did you regularly participate in sports and other vigorous physical activities (excluding walking and time spent in school physical education classes)?
   - During junior high school (JHS) or high school (HS) years—ages 12–18 yr (ask if 14 or older) _____
     ages 19–34 yr (ask if 21 or older) _____
     ages 35–49 yr (ask if 37 or older) _____
     ages 50+ yr (ask if 52 or older) _____
     past year _____
     past week _____
2. Did you ever compete in an individual or team sport (not including any time spent in sports performed during school physical education classes)? If yes,
   - How many total years did you participate in competitive sports? _____
3. Have you ever had a job that required physically demanding work? If yes,
   - How many physically active jobs have you ever held? _____
   - What is the total number of years that you have worked in these physically demanding jobs? (sum of years spent in jobs mentioned above) _____
4. Have you ever spent any time confined to a bed or a chair for greater than 1 month as a result of an injury or an illness? If yes,
   - How old were you when you first became confined to a bed or chair? _____
   - How many months did this confinement to a bed or chair last? _____
5. In general, about how many hours per day did you spend watching televison?
   - During your childhood years _____
   - JHS/HS years—ages 12–18 yr (ask if 14 or older) _____
     ages 19–34 yr (ask if 21 or older) _____
     ages 35–49 yr (ask if 37 or older) _____
     ages 50+ yr (ask if 52 or older) _____
     past year _____
     past week _____

Reliable test? (1, yes; 0, no) _____

Activity survey—occupational activities

Occupational activity—past year		Walk or bike to work?	Job schedule (average of past year)			Job activities At work, is most of your time spent sitting or performing light activities? If not, how is it usually spent?		
Job title* List all jobs held over past year for more than 1 mo (account for full 12 mo)	Age at start of job	min/day	mo/yr	days/wk	h/day	Hours per day spent in: Light activity	Moderate activity	Hard activity

*If unemployed/retired or housewife during all or part of the past year, list as such, and probe for job activities of a normal 8-hour day (excluding activities).

*More detailed instructions for questionnaire administration are available from A.M.K.

Activity survey—occupational activities

Occupational activity—historical Job title† List all other jobs held during lifetime for more than 1 year	Age at start and finish of job		Walk or bike to work?	Job schedule (average schedule)			Job activities At work, is most of your time spent sitting or performing light activities? If not, how is it usually spent? Hours per day spent in		
	Start	Finish	min/day	mo/yr	days/wk	h/day	Light activity	Moderate activity	Hard activity

†If more than 4 jobs, list the 4 jobs held the longest.

Light activities (includes standing, slow walking, and all sitting activities): sitting, standing, light cleaning (ironing, cooking, washing, dusting), driving a tractor or harvester, slow leisure walking.

Moderate activities (includes most indoor activity): carrying light loads (5–10 lb), continuous walking, heavy cleaning (mopping, sweeping, scrubbing, scraping), gardening (planting or weeding), painting/plastering, plumbing/welding, electrical work.

Hard activities (includes heavy industrial work, most outdoor construction, heavy farming): carrying moderate to heavy loads, shoveling, heavy construction, farming (hoeing, digging, mowing), digging ditches, chopping (ax), sawing.

Activity survey—leisure activities

Leisure activity code	Age												Past year			Past week
	12–18 yr (7 yr total)			19–34 yr (16 yr total)			35–49 yr (15 yr total)			≥50 yr (___yr total)			No. mo	Times each mo	Hours each time	
	No. yr	mo/yr	h/wk	No. yr	mo/yr	h/wk	No. yr	mo/yr	h/wk	No. yr	mo/yr	h/wk				No. hr

List codes of all activities done more than 10 times during lifetime.

Activity codes

01 School physical education	09 Skating (roller or ice)	17 Weight lifting
02 Running for exercise	10 Football/soccer	18 Calisthenics
03 Swimming (laps)	11 Racquetball/handball	19 Walking for exercise
04 Bicycling	12 Horseback riding	20 Hiking through mountains
05 Softball/baseball	13 Hunting	21 Rodeo
06 Volleyball	14 Fishing	22 Other _____
07 Bowling	15 Dancing	23 Other _____
08 Basketball	16 Gardening or yardwork	