The role of exercise in glycaemic control and inflammation in overweight/obese individuals with and without type 2 diabetes

by

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A thesis submitted to Murdoch University to fulfil the requirements for the degree of Doctor of Philosophy in the discipline of Exercise Science and Exercise Immunology

Perth, Western Australia 2018
Author’s Declaration

I declare that this thesis is my own account of my research and contains as its main content work which has not previously been submitted for a degree at any tertiary education institution.

______________________________
Aaron Raman
Statement of Contributors

I declare that this thesis is composed of my own research and work. I was involved in the conception of the work presented in each chapter; reviewing the literature for each chapter; writing the first draft of each chapter; interpreting the data in each chapter; and, I was solely responsible for all data collection in each chapter.

In addition, I claim the majority of authorship for each article presented in this thesis. In doing so, I declare that the co-authors as recorded below contributed to the relevant article by way of critically analysing and commenting on that article, as necessary, so as to contribute to its interpretation. More specifically, Associate Professor Fairchild, Associate Professor Peiffer, Professor Hoyne, Dr Currie and Mr Lawler contributed their expertise in relation to the analysis and report of the quantitative data present in Chapters Four, Five and Six. Each author provided their final approval of the relevant article prior to journal submission.

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Publication and Award

Chapter Two

Chapter Four

Chapter Five

Chapter Six

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Abstract

Evidence suggests the chronic low-grade inflammation associated with obesity increases the risk of developing type 2 diabetes mellitus (T2DM). Increased rates of exercise participation has been shown to reduce the risk of progression from overweight/obese to T2DM. Paradoxically, exercise results in an acute inflammatory response. The purpose of the research herein was to characterize the response in key inflammatory markers and glycaemia to acute exercise and short-term training in sedentary, overweight individuals and individuals with T2DM. Glucose tolerance and the interleukin-6 (IL-6) receptor pathway were assessed following acute (1h and 25h post-exercise) continuous exercise of moderate intensity (CME), and compared with a work- and duration-matched bout of high intensity intermittent exercise (HIIE; Chapter 4). There was an improvement in glucose tolerance immediately but not 25h post-exercise, concomitant with increased activity via the IL-6 classical signalling pathway. However, there were no differences between exercise modes and no associations between glucose tolerance and IL-6 signalling markers. The response in matrix metalloproteinase (MMP)-2 and MMP-3, as well as osteopontin was assessed following acute exercise in chapter 5. Continuous glucose monitoring was performed the day prior to exercise (control), the day of exercise, and the day after exercise and showed no between-day or condition (CME vs. HIIE) differences. However, differences in MMP-3 and osteopontin were identified in a direction consistent with protection against metabolic and cardiovascular disease. Effect of short-term exercise training (12 consecutive days of treadmill exercise) on inflammation and glucose control were assessed in previously diagnosed individuals with T2DM (Chapter 6). There were improvements in glycaemic control and global inflammation status post-training. Acute changes in pro- and anti-inflammatory cytokine responses following the first and last exercise session were also observed. The significance of these findings and future direction of research are discussed in chapter 7.
Acknowledgements

Completing a PhD in the field of exercise immunology and its relationship with Type 2 Diabetes was always the ‘next step’ I needed to take to contribute to making the world a healthier place. To Associate Professor Timothy Fairchild, I am so thankful to have had this opportunity to work with you and to learn from you. Your knowledge and passion in research has shaped me to become the person I am today. Thank you for your time and mentoring ability in decluttering new and complicated concepts which were foreign to me, now a clear picture in my head.

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<tr>
<td>APN</td>
<td>Adiponectin</td>
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<tr>
<td>AUC</td>
<td>Area Under the Curve</td>
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<td>BMI</td>
<td>Body Mass Index</td>
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<tr>
<td>CME</td>
<td>Continuous Moderate Intensity Exercise</td>
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<td>CGMS</td>
<td>Continuous Glucose Monitoring System</td>
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<td>CRP</td>
<td>C-Reactive Protein</td>
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<tr>
<td>DXA</td>
<td>Dual Energy X-Ray Absorptiometry</td>
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<td>FBG</td>
<td>Fasting Blasma Glucose</td>
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<tr>
<td>GLUT-4</td>
<td>Glucose Transporter 4</td>
</tr>
<tr>
<td>hsCRP</td>
<td>High Sensitivity C-Reactive Protein</td>
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<tr>
<td>HbA1c</td>
<td>Glycated Haemoglobin</td>
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<tr>
<td>HIIE</td>
<td>High Intensity Intermittent Exercise</td>
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<td>HRmax</td>
<td>Heart Rate Max</td>
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<tr>
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<td>Interleukin 1 Beta</td>
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<td>IL-1ra</td>
<td>Interleukin 1 Receptor Antagonist</td>
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<td>IIL-15</td>
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<td>Oral Glucose Tolerance Test</td>
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<td>Osteopontin</td>
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<td>Postprandial Glucose</td>
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<td>RPE</td>
<td>Rating of Perceived Exertion</td>
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<td>sIL-6R</td>
<td>Soluble Interleukin-6 receptor</td>
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<td>sGP130</td>
<td>Soluble Glycoprotein 130</td>
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<td>T2DM</td>
<td>Type 2 Diabetes Mellitus</td>
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<td>TGF-β</td>
<td>Transforming Growth Factor-Beta</td>
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<tr>
<td>Tregs</td>
<td>Regulatory T Cells</td>
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<td>Tumour Necrosis Factor Alpha</td>
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<td>VAT</td>
<td>Visceral Adipose Tissue</td>
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<td>VO_{2peak}</td>
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Chapter 1  Introduction

Obesity and physical inactivity are risk factors associated with the development of type 2 diabetes mellitus (T2DM) [1, 2]. The adipose tissue accumulation characteristic of obesity is linked with the onset of insulin resistance [3], both centrally (liver) and peripherally (skeletal muscle, adipose tissue) [4]. The compensatory hyperinsulinemic response augments the insulin resistance and in the longer-term leads to greater glycaemic impairment, consequently, increasing the risk of T2DM [5].

In lean healthy individuals, adipose tissue is predominantly infiltrated by alternatively activated macrophages which results in a pervasive anti-inflammatory environment, supported by regulatory T cells [6]. However, obesity facilitates a shift in the state of these infiltrated macrophages towards a classically-activated state, increasing the secretion of pro-inflammatory markers and reducing the suppressive capacity of regulatory T cells and anti-inflammatory cytokines such as Interleukin 10 (IL-10) [7, 8], influencing insulin signalling and glucose homeostasis [9]. Acute phase protein such as C-reactive protein (CRP) and pro-inflammatory cytokines such as tumour necrosis-alpha (TNF-α) and interleukin-6 (IL-6) have consistently been associated with obesity and T2DM [10-13]. The production of TNF-α in adipose tissue is implicated with increased insulin resistance in mice [14]. Specifically, neutralization of TNF-α with a soluble TNF-α receptor resulted in improvements in insulin-resistance in mice [15], and that TNF-α mediated insulin resistance by impairing proteins in the proximal insulin signalling pathway [15]. Given the importance of the insulin-stimulated translocation of glucose transporter-4 (GLUT4) to the plasma membrane in tissues such as liver, adipose tissue and skeletal muscle for glycaemic control, this impairment leads to prolonged periods of hyperglycaemia [16]. The production of TNF-α is also of importance as it induces the production of IL-6 which is associated with increased glucose intolerance and reduced insulin sensitivity in pro-inflammatory environments [17, 18]. Furthermore, the production of IL-6 induces the circulating concentration of CRP [10] which is also associated with obesity [12], which suggests to not only be a traditional risk marker for cardiovascular disease but also T2DM [17, 19]. In addition to these well-established inflammatory markers, a growing list of inflammatory mediators such as adiponectin, matrix metalloproteinases and osteopontin have been implicated in the adaptation to the pro-inflammatory state associated with obesity-related diseases [20-26].

Regular exercise has proven to be an effective tool in managing glycaemic control when compared to glucose lowering drugs in T2DM [27, 28]. In addition to insulin, exercise promotes glucose uptake in the muscle through the translocation of GLUT4 transporters from
its intracellular pool to the plasma membrane. Indeed, positive health outcomes are observed following long-term exercise training including reduced adiposity, improved glucose tolerance as well as reduced levels of pro inflammatory cytokines [29]. High-intensity intermittent exercise, comprising several ‘bursts’ of intense efforts interspersed with periods of lower-intensity exercise is an area of research that has received increasing attention due to it being perceived as being more enjoyable than continuous moderate-intensity exercise [30] and despite the reduced time-commitment relative to continuous moderate-intensity exercise, it has been found to be at least equivalent in improving aerobic capacity [31]. Additionally, various research groups have identified improvements in glycaemic outcomes following acute high-intensity intermittent exercise in individuals with [32, 33] and without type 2 diabetes [34], although not when compared to moderate-intensity exercise [35]. A recent review of high-intensity intermittent exercise on glucose control has highlighted its effectiveness in patients with and without type 2 diabetes [36]. In addition to acute exercise, improvements in glycaemic control have been reported following high-intensity exercise training, even in the short-term (≤ 2 weeks) [32, 33, 37, 38]. Aside from exercise intensity, previous meta-analysis has also reported the importance of exercise volume (frequency and duration) to be a significant factor in its effectiveness in improving glycaemic control [27]. With this in mind, a number of short-term training studies have assessed the glycaemic responses to consecutive exercise sessions for periods of 10 days [36, 39-41], and thereby increasing the frequency of exercise in this short period, and reported improved glycaemic control. A review of exercise and glycaemic control is covered in Chapter Two.

Whilst exercise training results in glycaemic improvements, exercise training similarly demonstrates large immune-modulating effects, and specifically, improvements in reducing chronic inflammatory markers such as CRP, IL-6 and TNFα [42]. Long term (≥ 3 months) exercise training has been found to improve CRP [43], as well as reduce pro-inflammatory and increase anti-inflammatory markers associated with the development of cardiovascular disease and type 2 diabetes [29, 43, 44]. Additionally, the effects of exercise on changes in the cellular immune compartments have been more recently assessed, with regulatory T-cells being identified as important cells due to the suppressive anti-inflammatory nature of these cells in down regulating the pro-inflammatory tissue environment [6, 45]. While a large body of evidence exists which has examined the effects of exercise on glycaemic control and immune responses independently, there is currently only limited research assessing the role exercise on glucose control concomitant with detailed inflammatory responses. Chapter three will therefore review the extant literature on exercise, inflammation and T2DM from an immune perspective.
Chapter Four investigated the role of acute exercise on glycaemic control and the subsequent changes in the IL-6 related inflammatory markers. Although a positive association between resting IL-6 and a risk for type 2 diabetes exists [46], an increase in IL-6 following exercise has been implicated in controlling glycaemia where its response has been shown to associate with increased exercise intensity [47]. The relationship between IL-6 and glycaemic control may be a consequence of the IL-6 distinct pathways with its soluble receptors, IL-6 receptor (IL-6R) and soluble glycoprotein (sgp130). This chapter assessed the role of exercise intensity on glycaemic control as well as the acute response to IL-6 and its receptors. Specifically, overweight sedentary adults performed either high-intensity intermittent exercise or continuous moderate-intensity exercise (cross-over design) and glycaemia and IL-6 related responses were assessed 24 h prior to exercise, immediately post-exercise, 1 h post-exercise, and 25 h post-exercise. Glycaemia was assessed following an oral glucose tolerance test which was completed to calculate glucose area under the curve (AUC) between each trial and the IL-6 related pathways were measured to assess concurrent changes associated with glucose tolerance. The exercise protocols in this study were matched for workload as well as exercise duration.

Using the same design and participants from Chapter Four, the purpose of Chapter Five was to assess the role of exercise intensity on diurnal glycaemic control and novel inflammatory markers associated with the initial stages of adipose-tissue expansion and T2DM. In this vein, the matrix metalloproteinases (MMPs) have now been shown to play potentially important roles early in obesity-related disease progression [48-50] and appear to be differentially expressed in clinical populations, with increased MMP-2 concentrations in obese patients [22]; increased MMP-2 concentrations [21] and decreased MMP-3 concentrations in patients with type 2 diabetes mellitus (T2DM; [23]). Osteopontin (OPN) on the other hand is a potentially important regulator of inflammation, obesity and diabetes [24], playing a particularly important role in macrophage movement and accumulation [51] and stimulating expression of MMP-2 [52]. The purpose of this chapter therefore, was to determine the acute (pre-, immediately post-, 1 h post- and 25 h post-exercise) effects of exercise on the inflammatory markers MMP-2, MMP-3 and OPN, and concomitantly assess changes in the 48 h glucose response to two different types of exercise in overweight and sedentary individuals using continuous glucose monitoring.

Chapter Six extends the findings from Chapters Four and Five, by way of recruiting individuals previously diagnosed with T2DM. Additionally, this chapter sought to disentangle the inflammatory responses to an acute exercise bout, in these sedentary individuals and the training responses. To this end, this chapter adopted a short-term training program, consisting
of 12 training sessions performed on successive days. To distinguish the acute and training responses, inflammatory cytokine (unstimulated and LPS-stimulated cytokine) responses to the first and last (12th) training session were assessed. The panels of cytokine included a range of anti- and pro-inflammatory cytokines including IL-10, IL-6, and TNFα, amongst others. To assess the impact of the training intervention, glycaemic control was assessed before and 48 h after the last training session by completion of an oral glucose tolerance test. Additionally, an alternative measure to glycated haemoglobin (HbA1c), fructosamine was also measured at these time points which is more appropriate in capturing short term (around two weeks) changes in glycaemic control [53]. With exercise training previously shown to be of anti-inflammatory nature [29, 44], we also measured regulatory T cells to determine if production of these cells were increased following exercise training which are responsible for promoting the production of anti-inflammatory cytokines, in addition to maintaining immune homeostasis [54] and its potential role in glucose control [55, 56].

Significance of the thesis

The contribution of the immune system in development of obesity-related disease, and specifically T2DM, has long been acknowledged. In the past few years, the importance of particular T-cell subsets such as the regulatory T-cells in obesity-induced inflammation have become better understood and novel cytokines in the role of adipose tissue development been identified. Concomitantly, the role of exercise in improving glycaemic control in individuals with T2DM is now universally acknowledged, and the underlying mechanisms better understood. The role of exercise in modulating components of the immune system have also evolved rapidly in the last decade, with research shifting towards a better functional understanding of the immune-changes in response to both acute and training responses. Much work in these fields has occurred in isolation, focusing on the individual effects of exercise on either glycaemic control or inflammation, but only limited research exists which has attempted to bridge these fields with a view to gaining a deeper understanding on their interaction. The work in this thesis aims to further our understanding of the inter-relationships between the immune-modulating effects of exercise and the glycaemic responses to exercise, in sedentary and overweight individuals with or without T2DM.
Chapter 2  Exercise, glucose transport and glucose regulation: A brief review

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The maintenance of glycaemia within a physiological range is not trivial, with postprandial glycaemic fluctuations occurring on the background of fasting glucose concentrations. Poor glycaemic control is associated with increased risk of cardiovascular disease [57, 58], renal disease [59], non-traumatic amputations [60] and all-cause mortality [61]. Unfortunately the prevalence of poor glycaemic control, either as type 2 diabetes mellitus (T2DM), impaired fasting glucose (IFG) or impaired glucose tolerance (IGT) is increasing globally; where an estimated 347 million people have diabetes, 90% of whom have T2DM [62].

The benefit of longer-term exercise training on glycaemic control (assessed by glycated haemoglobin [HbA1c]) is well established [63-65]. Improved glycaemic control is primarily attributed to the improved insulin-mediated glucose disposal, either through increased insulin sensitivity or insulin responsiveness [33, 34, 38, 66-68] rather than lower fasting glucose concentration [69]. While the general consensus clearly outlines a beneficial role of exercise with respect to glycaemic control, variability regarding the magnitude of benefit on glycaemic control exists. This variability may be due to the study population, the duration of the intervention, the type of exercise adopted or the outcome measure being used. The purpose of this review is to synthesise the information garnered from classic in vitro and in situ glucose uptake studies, and then apply this information to help explain the more recent clinical findings in response to exercise. The role of exercise intensity and duration will be a key focus of this review.

2.1 Glucose Transport

Cellular uptake of glucose occurs through facilitated diffusion using a carrier protein from the glucose transporter (GLUT) family. Glucose transporter type 1 (GLUT-1) is ubiquitously distributed and does not change in response to hormonal or other stimuli [70, 71]. For this reason, GLUT-1 is primarily responsible for glucose transport under basal conditions. The GLUT-4 on the other hand, are present primarily in adipose cells [72, 73] and striated (skeletal and cardiac) muscle cells [16], and translocates to the cell membrane from intracellular storage sites [74]. The translocation of GLUT4 from its intracellular compartments to the sarcolemma and t-tubules may be initiated by insulin binding [75], or in the case of muscle cells, in response to contraction (Figure 2.1;[76-80]).
Increasing doses of insulin results in increased recruitment of GLUT4 transporters to the cell membrane [81]. Accordingly, GLUT4 plasma membrane content increases with increasing contraction rates [82]. Insulin has been shown to increase the GLUT4 plasma membrane expression by up to a maximum ~7-fold (1mU/ml), whilst muscular contractions may maximally increase (10 Hz for 5 min) the GLUT4 plasma membrane expression up to ~4-fold [82]. When maximal insulin stimulation (1mU/ml) and muscle contractions (10 Hz for 5 min) were combined, a 9.3-fold increase in the GLUT4 plasma membrane expression was observed (versus the 7-fold and 4-fold increase by insulin and contraction alone, respectively) [82]. This additive effect may be explained by the existence of discrete intracellular GLUT4 pools, which are mobilized via discreet mechanisms [74, 77, 82]. Of relevance, GLUT4 protein content varies between muscles comprising different muscle fibre compositions [83], and the recruitment of GLUT4 to the cell membrane in response to either insulin or contraction has also been shown to vary in Male Wistar rats. Specifically, contraction was shown to elicit greater GLUT4 translocation in the flexor digitorum brevis (predominantly type 2a fibres) muscle, while Soleus (predominantly type 1 fibres) demonstrated greater GLUT4 recruitment with insulin (i.e., Soleus) at the same relative insulin concentration and contraction protocol [83]. Of significance to the present review, is the observation that in individuals with T2DM, exercise training has been shown to increase the total GLUT4 content in skeletal muscle [41] and adipose tissue [73].

![Figure 2.1](image-url)

**Figure 2.1.** Glucose Transporter number of subcellular membrane fractions from skeletal muscle. Data derived from [76].
Increased GLUT4 content in the cell membrane is associated with an increased rate of glucose transport (Figure 2.2; [78, 82, 84-86]). Indeed, the increased glucose transport in response to insulin [75] and contractions [87] as well as their additive effects (suggesting independent mechanisms) [88] were established prior to the identification of GLUT4 (albeit a transporter protein was known to be involved [75]), due to the relatively easier method of assessing glucose transport. Using in vitro models, contraction-induced glucose transport was increased 1.5-fold, 1.6-fold, 2.7-fold, 3.1-fold and 4.7-fold over basal glucose transport at contraction frequencies of 1 Hz, 2.5 Hz, 5 Hz, 7.5 Hz and 10 Hz, respectively; while GLUT4 content was increased by 2.5-fold and 4.1-fold at contraction rates of 5Hz and 10Hz, respectively [82]. When the frequency of contraction was increased to 25 Hz, there was no additional glucose uptake, indicating a plateau and that the maximum rate of glucose uptake was ~60% of a supra-maximal (1mU/mL) concentration of insulin [82]. Since these earlier experiments, a number of additional studies now support the increased glucose transport associated with contractions and insulin, and the additive effect when compared to each stimulus independently [86, 88-94].
With respect to contractions, increases in glucose transport have not only been seen with increases in the intensity of contractions, but also the duration of contractions [95-97]. These increased rates of glucose transport persist into recovery following exhaustive exercise, wherein 10-min post-exercise the rate of glucose transport is ~4.7-fold higher than pre-exercise; 30 min post-exercise the rate is ~3.3-fold higher than pre-exercise; 60-min post-exercise the rate is ~2.6-fold than pre-exercise; and 180-min post-exercise the rate is ~1.5-fold higher than pre-exercise [98].

**Figure 2.3.** Glucose uptake and the effect of insulin. Control group was not exercised; Postexercise group completed 45 minutes of treadmill running at 18m/min, with the last two minutes completed at 31m/min. Results are means ± SE with n=2-13 observations per data point. ° p < 0.05 when compared with control values [99].
2.2 Insulin sensitivity and responsiveness

Insulin responsiveness refers to the rate of glucose transport associated with a maximally effective insulin concentration (i.e., maximal glucose transport via insulin); while insulin sensitivity refers to the concentration of insulin required to achieve 50% of this maximal effect on glucose transport [100]. A classic example of improved insulin sensitivity following exercise was demonstrated by Richter and colleagues (Figure 2.3 [99]). It is noteworthy that no increase in glucose transport post-exercise was observed (as would be expected given the GLUT4 translocation with contractions), however, animals were provided *ad libitum* food access between exercise cessation and the perfusion experiments which were conducted in the subsequent few hours [99].

The duration of increased insulin sensitivity following exercise may persist from 3 hours to 48 hours and is dependent on the dietary status [99, 101, 102] and likely affected by the exercise performed (duration, intensity, type). In rats fed a carbohydrate-free diet post-exercise, the exercise-stimulated increases in glucose uptake (7.8-fold above non-exercised levels) were still largely present 18h later (3.5-fold increase above non-exercised levels), however, in rats fed a 60% carbohydrate diet the exercise-stimulated glucose uptake was no longer present (1.1-fold increase above non-exercise levels) [103]. This indirectly aligns with the earlier findings of Richter and colleagues [99], given the lack of difference in glucose transport post-exercise when animals were provided food in this period.

It is important to note that despite the extent of evidence from *in vitro*, *in vivo* and *in situ* studies demonstrating significant increases in glucose transport (measured by 3-MethylGlucose [3-MG]) following exercise or contractions, albeit to varying rates of glucose transport, some *in vitro* studies have reported no increase in glucose transport following contractions [89, 100, 104]. This discrepancy led to the identification that serum, and specifically a factor within serum, was critical for the increased glucose uptake following muscle contractions [89, 100]. For instance, glucose transport (epitrochlearis muscle; 3-MG) in the presence of insulin was i) increased 0.29±0.06 µmol.ml⁻¹.10min⁻¹ at rest; ii) increased 0.86±0.10 µmol.ml⁻¹.10min⁻¹ 3.5h following swimming; iii) increased 0.19±0.05 µmol.ml⁻¹.10min⁻¹ 3.5h following *in vitro* contractions (performed 15-min post excision while immersed in Krebs-Henseleit buffer; KHB) [89]. A follow-up experiment in the same study showed insulin sensitivity was largely retained 3.5h following the *in vitro* contractions, when performed immediately post-excision (0.43±0.04 µmol.ml⁻¹.10min⁻¹) versus a 15-min incubation in KHB buffer prior to contractions (0.18±0.03 µmol.ml⁻¹.10min⁻¹), although insulin sensitivity was not to the same degree as observed *in situ*. This rapid degradation in the
contraction-stimulated increase in insulin sensitivity associated with incubation in KHB buffer, suggested an important biological factor facilitating this increased sensitivity is rapidly lost. However, the exact property or molecule of the serum factor remains unknown; other than being a protein [100].

2.3 Tissue specific glucose transport in humans

The measurement of glucose transport in humans is complex and relies primarily on tracer-labeled glucose (such as $[^{13}\text{C}]$ glucose, $[^2\text{H}]$glucose) [105, 106], which when coupled with tissue biopsies [107], measures of arterio-venous glucose (AVg) difference [108], or imaging techniques (such as dynamic positron emission tomography [109] and magnetic resonance spectroscopy [110]) allow specific tissue/site glucose uptake rates. The findings in humans during 40 min of continuous exercise is consistent with animal work, demonstrating increased glucose uptake with increasing (up to ~80% VO$_{2\text{peak}}$) exercise intensity [111, 112]. The greater recruitment of total muscle fibres and in particular, the greater recruitment of fast-twitch fibres, likely underpin the higher rates of glucose uptake [111, 113], although there does appear to be a high degree of heterogeneity in the glucose uptake rates in fast-twitch muscles of humans [114]. Furthermore, the relationship between exercise intensity and glucose uptake is also of importance when applied to disease (T2DM) such that a greater reduction in plasma glucose was evident with increased intensity, reinforcing the significance of exercise for glucose control [115]. In addition to exercise intensity, exercise duration is associated with an initial (~30 min) rapid increase in glucose uptake [112] and a subsequent slower increase or plateau in glucose uptake over the next 60-90min [87, 115]. Insulin secretion is suppressed during exercise in a dose-dependent manner [116], wherein high intensity exercise is associated with increased leg glucose uptake, strongly suppressing insulin secretion and its circulating concentration [117-119].

2.4 Changes in systemic plasma glucose post-exercise: Looking beyond skeletal muscle

The beneficial effect of acute exercise on insulin responsiveness and sensitivity in muscle tissue has been confirmed during euglycaemic clamps performed prior to, and 1h and 24h subsequent to a 60 min cycling bout [120]. However, despite this localised improvement in glucose uptake, systemic postprandial glucose (PPG) tolerance remains either unchanged
increases [120, 125-129] or decreases [130-132] in the hours following an acute bout of moderate- and high-intensity exercise. The disparity between peripheral and systemic glucose concentrations may be due to an increased rate of hepatic glucose release, which continues for approximately 30 min after cycling in overweight/obese individuals with NGT [133] as well as a concomitant decrease in insulin sensitivity in tissues other than the contracting musculature [134]. Although in healthy middle-aged individuals, the glucose-AUC was shown to be highest (worst) immediately post-exercise, lowest (best) the day following exercise, and then demonstrated a progressive increase in the subsequent days, although even after 7 days, were still not as high as that experienced immediately post-exercise [130]. Since systemic plasma glucose concentrations are critical to successful management of individuals with impaired glucose tolerance, impaired fasting glucose and/or T2DM [64, 135], the underlying mechanisms explaining this apparent discrepant finding between increased localized glucose uptake and systemic glucose concentrations requires clarification.

Systemic plasma glucose concentration reflects the balance between the rate of glucose appearance (Rₘ) and rate of glucose disappearance (Rₜ). This balance is complicated by the multiple factors contributing to Rₘ and Rₜ respectively. For instance, glucose Rₘ in the fasted period is principally governed by total hepatic glucose release, while in the postprandial period is governed by total hepatic glucose release and the glucose in the portal vein (dietary glucose absorbed from the small intestine) remaining following the first-pass; while Rₜ is the sum total of glucose uptake by all cells of the body. The balance between Rₘ and Rₜ becomes more complex with acute exercise, since both Rₘ and Rₜ may be affected, specifically i) acute exercise may increase- in orders of magnitude- glucose uptake into the exercised/contracted muscles, whilst concomitantly decreasing glucose uptake in other tissues of the body; ii) acute exercise may increase net hepatic glucose release and rate of glucose absorption from the small intestine [133]. The underlying mechanisms regarding these changes are discussed below, with specific emphasis on free fatty acids (FFA), epinephrine, glucagon, growth hormone and cortisol. A previous review has also proposed a model which discusses the potential mechanisms of Akt Substrate (AS160) which may explain the increased post-exercise glucose uptake by acting as a potential trigger, memory element and/or mediator of increasing insulin sensitivity [136].

2.4.1 Gluoregulatory effects of key Hormones: Epinephrine, glucagon, growth hormone and cortisol

Increased hepatic glucose output is essential in sustaining exercise capacity and preventing hypoglycaemia [137, 138], and the increased glucose output continues for up to 45-60 min
into the recovery period [133]. This increased glucose output coincides with increases in glucagon, catecholamine and cortisol concentrations, and decreases in insulin concentration [138, 139]. Of these hormones, single infusions of epinephrine and glucagon are associated with increases in plasma glucose during rest, with glucagon stimulating hepatic glucose output to the greatest effect, while cortisol appears to have negligible effect in isolation [96, 140-142]. In accordance, glucagon- in concert with the associated decrease in insulin concentration- has been shown to play the greatest role in increasing the hepatic glucose release observed during exercise [143, 144]. The role of the catecholamines, and specifically epinephrine on increased hepatic glucose output have been shown to be minimal via studies conducted with hepatic denervation [145], β- and α-adrenergic receptor blockade [146], and adrenalectomy [147]. It is noteworthy that the hormones appear to act synergistically, with the addition of epinephrine and glucagon being greater than either alone, and the addition of cortisol appears to prolong the action of these hormones on the liver [140].

While the effects of glucagon appear to be largely limited to the liver, epinephrine has been shown to play important roles in glucose clearance [148]. In perfused rat muscles, epinephrine (25 nM) significantly increases in GLUT4 content and glucose transport (22%-48%), wherein the greatest increase occurs in oxidative muscle (red gastrocnemius and soleus) when compared to muscles perfused without epinephrine [149]. In contrast, infusion of epinephrine (25 nM) together with insulin (20 mU/ml) partially inhibits the insulin stimulated glucose transport when compared to insulin infusion (20 mU/ml) alone, despite the persistent increase in GLUT4 plasma membrane content [149]. Oxidative muscle appears to be consistently more sensitive to epinephrine which is likely a result of the greater number of β2-adrenergic receptors [150]. Subsequent work found the inhibition by epinephrine (24 nM) persists at 50 mU/ml but not 100 mU/ml of insulin, even when the concentration of epinephrine was increased to 500 nM [151], suggesting that very high levels of insulin overcome this inhibition by epinephrine. To assess the mechanism associated with this downregulation, insulin receptor substrate-1 associated phosphatidylinositol 3-kinase (PI3K) activity was assessed and found to be suppressed with addition of epinephrine [151], although this inhibitory effect was ablated at higher insulin (100mU/ml) concentration.

The effect of epinephrine in humans has been assessed during a euglycaemic clamp performed with or without epinephrine, along with the β1-adrenergic receptor antagonist metoprolol, or the β1- and β2-adrenergic receptor antagonist propranolol [152]. The major findings (Figure 2.4) were i) glucose Ra decreased substantially with the addition of insulin, while Rr concomitantly increased; ii) Addition of epinephrine with the insulin, resulted in an increased Ra relative to insulin infusion alone, while the Rr of glucose was reduced; iii) this effect was
exerted through the β₂-adrenergic receptors as evidenced by propranolol being able to ablate the insulin-antagonizing effect of epinephrine while this inhibitory effect persisted in the presence of metoprolol [152].

**Figure 2.4.** Rate of glucose appearance (Rₐ) and disappearance (Rₐ) during steady state glucose infusion rate. A. Measures taken before the clamp (PLA), during the clamp at low (Low Ins; 4mU/l) and high (High Ins; 51mU/l); B. Measures taken before the clamp (PLA), during the clamp at low (Low Ins; 4mU/l) and high (High Ins; 51mU/l) insulin infusion rates with addition of epinephrine (Low Ins + A; High Ins + A); C. measures taken during the Low insulin clamp (40 mU.(m²)⁻¹.min⁻¹; Ins), epinephrine only (A), insulin with epinephrine and propranolol (Ins+A+Pro), insulin with epinephrine and metoprolol (Ins+A+Met); D. Measures taken during the High insulin clamp (1200 mU.(m²)⁻¹.min⁻¹; Ins), epinephrine only (A), insulin with epinephrine and propranolol (Ins+A+Pro), insulin with epinephrine and metoprolol (Ins+A+Met).
An increase in growth hormone is also evident following exercise [143] concomitant with an increase in exercise duration and intensity [139, 153]. Growth hormone has been implicated in the development of insulin resistance, which was identified as being due to both increased hepatic glucose output and reduced glucose clearance [154]. While growth hormone does appear to promote gluconeogenesis and increased hepatic glucose production [155, 156], this effect seems minimal [141]. In muscles, increased concentration of growth hormone has been shown to down regulate glucose transporters (GLUT1 and GLUT4) and glucose uptake [157]. Increases in growth hormone and epinephrine are also accompanied with increased lipolysis, and consequently, increased free fatty-acid (FFA) concentrations [139, 154, 155, 158-160]. The increase in circulating FFA interferes in glucose uptake via both insulin-dependent [161, 162] and independent effects via decreased cellular glucose utilisation [116, 163-165], which can act synergistically [3]. During exercise in rats, increased concentration of FFA resulted in significantly higher blood glucose [166], while in humans, elevated plasma FFA concentrations following a high-fat diet resulted in increased blood glucose [167].

2.4.2 The role of exercise intensity

Increases in the concentration of catecholamines (epinephrine and norepinephrine), growth hormone and glucagon are positively associated with increases in exercise intensity and duration [168-171]. Likewise, utilisation of muscle glycogen increases with both intensity and duration of exercise [172-176] with plasma glucose utilization demonstrating a similar trend [172]. Accordingly, when matched for work, low-moderate intensity (40% $\mathrm{VO}_{\text{peak}}$) exercise has been shown to result in a similar GLUT4 recruitment to high-intensity (80% $\mathrm{VO}_{\text{peak}}$) exercise [177], and this translates to similar plasma glucose responses to an oral glucose tolerance test performed 1h [126, 129] and 24h post-exercise of low- or high-intensity [126]. Reductions in muscle glycogen concentration, have been associated with increased glucose uptake [115]. In longer-term training studies (6 months), total work has been shown to be more important than either intensity or duration alone [178].

More recently, interval-based training in the form of high-intensity intermittent exercise (acute) or high-intensity intermittent training (‘longer-term’) has gained increasing attention [31, 179]. Indeed, HIIE has been shown to decrease post-meal glucose AUC and reduce hyperglycaemia during the 24h following exercise as measured by a continuous glucose monitoring system (CGMS) in overweight/obese subjects with T2DM [32, 33]. Despite the improvement in 3h post-meal glucose AUC, the changes in postprandial glucose remains
unclear as 24h average blood glucose was not different [32]. Additionally, very few studies have adopted a comparator condition, with the initial studies adopting a single-arm, pre- and post-assessment design only [33] or comparing glucose changes to a no-exercise control condition [32]. Indeed, one study comparing the effect of interval-based training against moderate-intensity (75% VO2peak) exercise, showed greater improvements in insulin sensitivity with moderate-intensity exercise compared to sprint interval exercise in overweight/obese individuals; although an improvement in glucose AUC was not evident [35]. Since the initial landmark studies on high intensity interval exercise and high intensity intermittent training [33, 180], questions remain on whether this form of training is superior to a bout of work-matched continuous moderate-intensity exercise.

2.5 Short-term consecutive training

Meta-analyses have identified the benefits of chronic (>8 weeks) exercise-training on improvements in HbA1c [28, 179]. There is also evidence of improved glycaemic control following short-term (≤2 weeks; 3 to 10 sessions) exercise training [33, 34, 38, 40, 41, 67, 115, 181-185], despite large heterogeneity between studies.

Short-term high intensity training studies remain in conflict as mixed findings have been reported in the improvement of glucose AUC in non-T2DM individuals following two weeks of training. Although both training protocols comprised of Wingate exercise protocols (all-out cycling efforts for 30s), a reduction of glucose AUC following an oral glucose tolerance test was only evident in young non-obese individuals [67, 186], with no such improvement in overweight/obese males [187]. Despite this difference, both studies found short-term training to result in an improvement in insulin sensitivity, derived from insulin sensitivity indices. Interestingly, the improvement of insulin sensitivity was evident two to three days following exercise in the young individuals [67, 186], rather than 24 h following the last exercise bout in the overweight/obese males [187]. Additionally, a comparable study did not find improvements in insulin sensitivity, which may be explained by the lack of improvement in GLUT4 recruitment measured post-training [185], contrary to previous work where increased GLUT4 was reported after a week of exercise training [181]. The difference in training stimulus may also be considered as a limiting factor as the protocol implemented an 8-12s
active period followed by 80s of recovery which resulted in a lower training volume [185].

Similar short-term exercise training employing high intensity exercise has also been completed in individuals with T2DM [33]. The exercise protocol was completed at 90% HRmax rather than the exhaustive Wingate protocol, Little et al. demonstrated that six sessions of interval training (10 x 60s @ 90% HRmax) reduced both 24 h blood glucose concentrations as well as post-meal 3 h postprandial glucose [33]. Although insulin sensitivity was not measured, the improvement in glycaemic control was accompanied by increased GLUT4 protein of ~360% highlighting the importance of exercise intensity and volume [33]. With improvements seen only after six sessions over a two-week period, further research has investigated the impact of consecutive days of exercise training for up to seven days in individuals with T2DM [40, 41, 115, 182, 184, 188]. The consequence of seven days of consecutive training results in an increase training volume although these protocols varied between 50 to 70 minutes of exercise at 50% to 75% of VO2peak consisting of treadmill and cycling exercise, or both. The increase in training volume resulted in improved insulin sensitivity as demonstrated by increase insulin-stimulated glucose disposal [41, 188], as well as reduced insulin and glucose AUC [184]. Gorman et al. also demonstrated that the increased insulin sensitivity may be attributed to the increase in GLUT4 content following 7 days of exercise [41]. These examples have shown positive outcomes in insulin sensitivity and reduced glucose AUC, however, two studies [182, 183] failed to show improvements in glucose AUC, whilst Kang and colleagues did not find improvements in either glucose AUC nor insulin sensitivity following an oral glucose tolerance tests [115].

Currently, it is not known whether exercise intensity, exercise volume or a combination of increased exercise intensity and exercise volume is important in driving the improvements seen in insulin sensitivity and glucose tolerance.
2.6 Conclusion

Despite existing evidence suggesting improvements in insulin sensitivity and glycaemic control following exercise, there remains debate concerning the extent of improvement in glucose tolerance. This debate remains due to significant heterogeneity in existing literature, and methodological limitations such as a lack of a comparison group [32-34]; unmatched groups in exercise duration and intensity [115]; volume [177, 178]; and non-standardised glucose assessment via continuous glucose monitoring [33-35, 102] versus oral glucose tolerance test [130, 131, 133]. Work by Knudsen [133] provided an updated insight into the post-exercise meal effect on glucose tolerance which confirms the results of previous research [130, 131] in healthy individuals such that plasma glucose and glucose AUC was significantly increased post-exercise. This increase may be explained by the increased rate of glucose appearance following exercise together with an increased rate of glucose disappearance. Despite the increase in plasma glucose, insulin sensitivity was improved as measured by the insulin sensitivity index which highlights an interesting point that reduced glucose AUC was not evident together with this improvement. As suggested by the authors, the increase rate of appearance may be due to reduced uptake in hepatic glucose following increased intestinal absorption in splanchnic tissues. Further research is warranted to answer the limitations discussed including time and work-matched exercise groups, a suitable comparison group (moderate intensity exercise) over a training period, as well as the inclusion of relevant comparison populations during high-intensity interval training. What can be confirmed however is that plasma glucose is not consistently improved, and is instead increased following exercise in subjects who have normal glucose tolerance [130, 132, 133] and with T2DM [96].
Chapter 3  Low grade inflammation, exercise and type 2 diabetes: A brief review

This chapter will not be submitted for publication.
Obesity is characterised by the expansion of adipose tissue and is associated with an increased risk of insulin resistance [3] and a chronic inflammatory response originating from adipose tissue reaching systemic circulation [189]. As a consequence of obesity-induced insulin resistance, chronic elevated blood glucose concentration leads to the development of Type 2 Diabetes (T2DM; defined by glycated haemoglobin (HbA1c) ≥ 6.5%). Evidence suggests that increased adipose tissue contributes to a chronic state of low grade inflammation which is more evident in obese individuals than lean healthy individuals, representing a link between obesity, inflammation and T2DM [190, 191]. Although both subcutaneous and visceral adipose tissue are associated with increased metabolic risk factors [192], studies have shown greater importance of visceral adipose tissue on obesity related inflammation [193, 194].

Increased visceral adipose tissue leads to greater macrophage infiltration resulting in an increase of several pro-inflammatory markers such as Interleukin (IL)-6 and tumour necrosis factor alpha (TNFα) [7, 195], whilst elevated C-reactive protein (CRP) produced from hepatocytes is also elevated [196]. These markers have been previously associated with T2DM [46, 197]. The infiltration of macrophages can also be classed in at least two forms; anti-inflammatory ‘alternatively-activated’ (M2) macrophages which may be polarised to ‘classically activated’ (M1) macrophages which produce pro-inflammatory cytokines associated with insulin resistance [198].

An increase in obesity-induced pro-inflammatory marker, TNFα impairs insulin signalling is associated with insulin resistance, affecting glucose homeostasis [14]. Neutralization of TNFα in mice results in the attenuation of insulin resistance, further highlighting the direct relationship between inflammation and insulin resistance [14]. Additionally, elevated IL-6 is associated with an increased risk of T2DM through induction of CRP release which is also associated with the development of T2DM [10]. In addition to the positive association of IL-6 and CRP with T2DM, evidence has also identified a role of additional markers such as Matrixmetalloproteinases (MMPs) which is related to the production of TNFα [199], whilst IL-6 has been shown to mediate the expression of Osteopontin (OPN) which is elevated with obesity and positively correlated with fasting blood glucose [200]. Obesity and T2DM are also accompanied with a reduced anti-inflammatory profile, affecting factors such as adiponectin [201], IL-10 [202] and regulatory T cells [6, 203] which are crucial in maintaining immune homeostasis [204]. It is evident that inflammation is dysregulated in obesity and T2DM, thus an appropriate intervention that targets both obesity and inflammation, such as increased physical activity may be effective in the reducing the clinical effects of obesity-associated chronic inflammation.

Regular exercise is beneficial in the management of blood glucose, reducing the risk of T2DM [27]. Further to glycaemic control, acute exercise and exercise training also modulate the
immune system, demonstrated by changes in the balance of both anti-inflammatory and pro-inflammatory markers following exercise [29, 205, 206]. Although exercise stimulates an immune response, physical inactivity leads to the impairment of glycaemic control [207] which contributes to obesity and an increased risk of T2DM, consequently accompanied with chronic inflammation. Therefore, regular exercise is not only important in managing glycaemic control but may also contribute to exercise-induced improvements in chronic inflammation, especially in individuals who are at risk, or have previously been diagnosed with T2DM. Despite previous work highlighting the beneficial health effect of exercise, the understanding of the inter-play between exercise and chronic low-grade inflammation and the subsequent impact on T2DM remains limited. Since measurement of cytokines such as TNFα or IL-6 does not directly cause T2DM in humans, but are rather associated with T2DM, additional research is needed to understand the role of acute exercise and exercise training on the regulation of classic inflammatory markers such as IL-6, CRP, TNFα and IL-10, as well as related inflammatory markers including MMPs and OPN which have been reported to be elevated and associated with obesity and/or T2DM [21, 208]. Combining the measurement of inflammatory markers together with the assessment of glucose-related responses is crucial in determining the role exercise may have in regulating both the inflammatory response and any associated relationship with glucose homeostasis.

Although exercise and T2DM are accompanied with an inflammatory response which involve a large number of inflammatory mediators, this review will focus on selected markers including IL-1β, IL-6, IL-10, TNFα, CRP, adiponectin, OPN, MMPs and regulatory T cells. Of the relevant markers, the review will then discuss the impact of acute exercise on the inflammatory response and the beneficial role exercise training may have on regulating chronic inflammation experienced in obesity and/or T2DM. A combination of these markers were measured following acute exercise (Chapter Four and Five) in overweight/obese individuals and following short-term training in individuals diagnosed with T2DM (Chapter Six).
3.1 Inflammation, obesity and T2DM

3.1.1 Pro-inflammatory cytokine markers associated with T2DM

Studies have shown that a number of prominent inflammatory markers are elevated in T2DM including CRP, IL-1 beta (IL-1β), IL-6 and TNFα [10, 11, 46, 209]. Of relevance, the release of these markers are associated with increased risk towards the development of T2DM through the disruption of insulin signaling where the insulin receptor is phosphorylated by serine instead of tyrosine leading to the inhibition of downstream signal transduction, promoting insulin resistance [15, 209-211].

Adipose tissue has been found to be a large source of IL-6 [212]. The production of IL-6 has also been found to be affected by hyperglycaemia, both in vitro [213] and in vivo [214]. Since hyperglycaemia is a consequence of obesity and insulin resistance, an elevated concentration of IL-6 is correlated with increased BMI [215, 216], and is associated with the severity of glucose intolerance and reduced insulin sensitivity [17, 18]. Thus, the increase in IL-6 in the transition to obesity strongly supports the link between obesity and insulin resistance [217, 218], increasing the risk for development of T2DM [13, 219, 220]. Extending to the association with insulin resistance, the release of IL-6 induces the production of CRP [217], which is an important marker on vascular inflammation and atherosclerosis [221]. Plasma CRP is correlated with body fat percentage, BMI, fasting plasma glucose and 2-hour plasma glucose following a glucose tolerance test proving to be an important risk marker for not only cardiovascular disease but also T2DM [10, 17, 19, 46]. Previous work has found CRP levels to be inversely associated with insulin responsiveness [217], and significantly increased in individuals with impaired glucose tolerance and T2DM, compared to control subjects [19, 46, 197, 217]. Additionally, a positive correlation between IL-6 and CRP with impaired glycaemic control has been previously reported [17]. A progressive increase in the concentration of IL-6 and CRP were also found together with increased severity of metabolic syndrome and T2DM where they were lowest in non-diabetic individuals, intermediate levels in metabolic syndrome-negative T2DM and highest concentration in metabolic syndrome-positive T2DM subjects [219].

TNFα is a pro-inflammatory cytokine highly expressed in adipose tissue associated with insulin resistance and T2DM. Seminal work by Hotamisligil and colleagues highlighted the
The relationship between TNFα and insulin resistance through neutralization of TNFα with a soluble TNFα receptor in obese, insulin-resistant mice *in vivo*, which resulted in decreased insulin resistance [14]. Further evidence have since supported the overproduction of TNFα in obese human adipose tissue [222-224] particularly when compared to non-obese individuals [225]. The role of TNFα was further demonstrated by an inverse association between TNFα and insulin sensitivity demonstrated by reduced glucose disposal rate [224]. The main mechanism by which TNFα promotes insulin resistance, occurs by limiting phosphorylation of the insulin receptor and interfering with insulin action via TNFα-induced serine phosphorylation of IRS-1 [15, 226]. The concentration of TNFα in obese individuals has been demonstrated to reduce through weight loss following lifestyle modification with potential positive effects on insulin sensitivity and insulin resistance evident from reduced insulin area under the curve following a glucose challenge [227].

IL-1β, a major IL-1 family cytokine produced by macrophages [228], has previously been implicated in type 1 diabetes through the destruction of pancreatic β cells [229]. In addition to IL-6, TNFα and CRP, previous work has also reported elevated concentration of IL-1β in T2DM [11, 13, 46]. This pro-inflammatory cytokine is a significant marker of the development of T2DM, as the secretion of IL-1β has also been found to be promoted under high glucose concentration during *in vitro* exposure of IL-1β supporting that the similar hyperglycaemic environment experienced in T2DM may induce the production of IL-1β. Furthermore, *in vivo* pancreatic sections of type 2 diabetic subjects were presented with elevated IL-1β but not in nondiabetic control subjects [230], suggesting that the pancreas may be responsible for the synthesis and release of IL-1β [231]. Additionally, the increase of IL-1β in T2DM patients was also accompanied with reduced concentration of anti-inflammatory marker, IL-1 receptor antagonist (IL-1ra) *in vitro* [232] highlighting a bias towards a pro-inflammatory environment as a consequence of T2DM upsetting the balance of inflammatory cytokines. Thus, consistent evidence has highlighted that T2DM is accompanied with elevated concentration of pro-inflammatory markers including IL-6, CRP, TNFα and IL-1β. The release of these markers is induced with increased levels of adipose tissue which is a main source of these markers in addition to adipose-resident macrophages and crown like structures which reside in necrotic adipocytes which infiltrate into adipose tissue as a consequence of obesity [233, 234]. These inflammatory markers also extend further to associate with factors related to T2DM including hyperglycaemia, insulin resistance, increased BMI, impaired glucose tolerance and decreased insulin sensitivity.
3.1.2 Additional inflammatory markers associated with T2DM

In addition to the markers discussed above, three inflammatory markers associated with vascular disease and coronary heart disease; Matrix Metalloproteinase (MMP) 2, MMP3 and OPN have been previously linked with insulin resistance/T2DM [21, 208, 235, 236]. MMPs are enzymes responsible for the breakdown activity in vascular extracellular matrix remodeling during obesity-induced adipose tissue formation [48]. Their proteolytic activity plays a role in plaque rupture and destabilization in atherosclerotic lesions [237]. Together as a marker for vascular disease, the expression of increased MMP2, MMP3, MMP9 were previously elevated in obese individuals with and without T2DM when compared to healthy subjects [22, 23]. Goncalves et al. also highlighted that type 2 diabetic subjects presented higher levels of MMPs when compared to those without T2DM, recognizing MMPs as potential risk markers to consider for the development of T2DM [23]. Further work in cell culture of adipocytes and high fat fed mice demonstrated enhanced MMP-3 release which stimulated the release of TNFα and resulted in the inhibition of insulin-induced glucose uptake in adipocytes. However, neutralization of MMP3 inhibited TNFα secretion, indicating that increased MMP3 may promote insulin resistance through the release of TNFα [199]. Elevated concentrations of MMP2 and MMP9 have also been found under high glucose conditions in human umbilical vein endothelial cells in vitro, highlighting the significance of a hyperglycemic environment as experienced in individuals at risk of T2DM, in addition to vascular complications [238].

The formation of active MMP2 from pro-MMP2, can be induced by the release of OPN. OPN is a pro-inflammatory glycoprotein associated with bone formation and is abundant in atherosclerotic plaques, specifically in calcified plaques [239]. In addition to an increased risk of atherosclerosis [240], OPN is associated with obesity, evident from increased concentration in obese high-fat fed mice [241] and increased adipose tissue resulting from obesity in humans [208]. Further work has also identified that an increase in IL-6 is a significant mediator to the expression of OPN expression in macrophages [242]. Elevated OPN was found in obese individuals with [20, 208] and without [208] T2DM when compared to healthy individuals with normal glucose tolerance. This difference between populations was also highlighted from a positive correlation of OPN with fasting blood glucose in obese individuals when compared to a lean group [200]. Furthermore, increased OPN levels also correlated with an increased percentage of body fat, as well as physical inactivity and physiological variables such as reduced insulin, elevated QUICKI (insulin sensitivity index) and elevated CRP, highlighting the relevance of OPN in the development of T2DM [243].
3.1.3 Anti-inflammatory markers associated with T2DM

The concentration of the anti-inflammatory cytokines IL-10 and adiponectin in blood are inversely associated with the development of T2DM [244-247]. Adiponectin is exclusively secreted and synthesized by adipose tissue macrophages and then released into blood [248]. Low adiponectin levels are also associated with decreased insulin sensitivity, with the lowest levels seen in individuals with T2DM [249]. Furthermore, the production of adiponectin is reduced by the release of TNFα, IL-6 and CRP [248, 250], suggesting that the obesity-induced increase in pro-inflammatory markers is detrimental to the anti-inflammatory effect of adiponectin. The protective effects of adiponectin is suggested to be glucose- and immune-related, resulting in reduced blood glucose [251], increased insulin sensitivity [252] via the induction of tyrosine phosphorylation of insulin receptor [247], inhibition of pro-inflammatory cytokines TNFα and IL-6 [248], and stimulation of the production of anti-inflammatory marker, IL-10 [253, 254].

IL-10 can be produced by a range of cells including monocytes, macrophages and regulatory T cells, amongst other innate and adaptive immune cells [255]. A population based study revealed that low concentration of IL-10 was associated with an increased risk of T2DM [203]. IL-10 is a potent anti-inflammatory cytokine that inhibits the production of pro-inflammatory cytokines including IL-6, TNFα, and IL-1β [256, 257]. Thus, the suppressive function of IL-10 is crucial in maintaining and promoting an anti-inflammatory environment. The protective effect of IL-10 in glucose control was also evident in IL-10-treated mice following a high fat-fed diet, resulting in improved insulin signaling and sensitivity [198, 258] as well as the attenuation of macrophage infiltration in insulin resistant mice [259]. In contrast, limited production of IL-10 in murine models resulted in greater macrophage infiltration, contributing to a greater pro-inflammatory response [260, 261]. The suppressive function of IL-10 is also crucial to the function of regulatory T cells (Tregs) which produce IL-10 as a major suppressive factor [262]. Tregs (CD4+CD25+CD127- T cells) play an important role in the maintenance of self-tolerance and immune homeostasis [54]. Tregs can be generated in the thymus and then migrate to peripheral tissues, or alternatively can be induced from peripheral lymphocytes upon stimulation from cytokines [54]. Growing evidence have found that a reduced number of circulating Tregs in obese individuals is associated with obesity and T2D [202, 263-265] and is inversely correlated with BMI [6, 263] and HbA1c [202] which may contribute to insulin resistance [6, 55, 266]. Additionally, the reduction of Tregs in visceral adipose tissue has also been associated with decreased insulin sensitivity [55] and is also
accompanied with reduced immune-suppressive capacity and a skewed pro-inflammatory environment increasing the risk of insulin resistance [55, 263]. Tregs are important in the suppression of a pro-inflammatory environment such as TNFα-induced insulin resistance via secretion of IL-10 [198, 267]. Previous work has highlighted the importance Tregs such that depletion of Tregs in obese mice has been demonstrated to lead to increased insulin resistance in mice [55]. However, induction or the transfer of exogenous Tregs in mice resulted in improved glucose tolerance and insulin sensitivity highlighting the therapeutic potential of Tregs [55, 56].

3.2 Exercise and inflammation – intensity, mode and duration

Regular physical activity has an important role in the prevention of T2DM [268], cardiovascular disease [269] as well as all-cause mortality [270]. Exercise increases the rate of glucose uptake through the translocation of GLUT4 transporters from intracellular storage sites to the cell membrane to facilitate the transport of glucose into the muscle [271]. Increased GLUT4 expression results in local muscles to be more sensitive to the action of insulin improving insulin sensitivity [272, 273] and an additive effect in glucose uptake is evident if both exercise and insulin are present when compared to each stimuli independently [86, 90]. Previous meta-analyses have suggested that the beneficial health effect of exercise was more effective in reducing HbA1c in type 2 diabetics when compared to glucose-lowering drugs [27, 28, 274]. Interestingly, consistent with the increased rate of glucose uptake accompanied with increased exercise intensity, previous meta-analyses also support greater reduction in weighted mean difference of HbA1c with exercise training at higher intensities [179, 275]. However, it must be noted that others have found the exercise-induced reduction in HbA1c was associated with exercise volume, rather than intensity [179]. Overall this suggests that although it is not clear whether exercise intensity or exercise volume is superior when prescribing exercise, meta-analyses have identified similar or greater therapeutic benefit of exercise when compared to glucose-lowering drugs.

Acute exercise stimulates the production of pro- and anti-inflammatory markers where it is mediated by exercise duration, intensity and mode. Ostrowski et. al. demonstrated that acute exercise induces the release of IL-6, which gradually increased over time during treadmill running [276]. Further work following marathon running, highlighted that prolonged strenuous exercise induced a significant pro-inflammatory response of up to 128-fold in IL-6,
TNFα and IL-1β levels immediately following exercise, whereas anti-inflammatory cytokines IL-10 and IL-1ra peaked to a lesser extent immediately post exercise and 1-hour post exercise, respectively [204]. In addition to exercise duration, the inflammatory response is also driven by exercise intensity. A number of studies found that the production of pro- and anti-inflammatory cytokines including IL-6, IL-1β, IL-8, IL-10 and TNFα were increased together with increased exercise intensity [47, 277-279]. However, previous work in healthy individuals have found no differences in anti-inflammatory cytokines IL-4 and IL-10 [280]. Indeed, it may be plausible that the anti-inflammatory effect of exercise may be present at the adipose tissue level, without any effect on plasma inflammatory cytokines [206].

Further to the variables of exercise intensity and duration, the mode of exercise may also contribute to the degree of the inflammatory response. Nieman and colleagues have shown that downhill running (eccentric contractions) induced significantly greater concentration of inflammatory markers including IL-1β, IL-6, IL-10, TNFα and others when compared to concentric cycling [281]. Their results also demonstrated that downhill running induced greater level of muscle damage with elevated creatine kinase, myoglobin and delayed onset of muscle soreness[281]. This confirms the findings of previous work, where eccentric cycling induced greater creatine kinase and IL-6 when compared to concentric cycling [282]. However, although significant differences in cytokine response were found between modes of exercise, evidence suggests that exercise intensity may have a stronger influence on cytokine changes compared to muscle damage as the exercise-induced increase in stress hormones such as cortisol and norepinephrine may have a greater influence on the systemic release of cytokines rather than exercise-induced muscle damage [283]. Therefore, the inflammatory response between mode of exercise should be interpreted with caution as multiple factors may contribute to the magnitude of cytokine release.

### 3.3 Inflammation in T2DM – exercise-induced improvements

In addition to the improvements seen with exercise training on glucose control as reviewed in Chapter Two, a reduction in chronic inflammatory markers such as CRP [214, 284, 285] and IL-6 [11, 42, 46] is also evident. Several studies showed that, following 3 to 12 months of exercise training in T2DM patients, pro-inflammatory markers such as CRP, IL-1β, IL-18 and
TNFα were reduced (up to ~40%), whilst anti-inflammatory cytokines IL-4 and IL-10 increased (up to ~84%). These changes in cytokine levels were associated with reduced insulin resistance in T2DM patients [29, 44, 286]. In contrast, Dekker et.al found that while exercise training in obese men with and without T2DM for 12 weeks did result in a reduction in IL-6, there was no change in insulin sensitivity [287].

Whilst acute exercise results in an increased inflammatory response where most markers return to resting concentrations within 24 hours, regular exercise promotes an inflammatory response which reduces chronic inflammation in both chronic disease populations [288, 289] and in healthy adults [290]. The exercise-induced reduction in chronic inflammation may be driven by IL-6. Although IL-6 has been described as a pro-inflammatory marker primarily originating from adipose tissue and has a strong association with obesity and insulin resistance, previous work has also demonstrated its anti-inflammatory properties following exercise [291]. IL-6 is one of the first cytokines released into circulation which increases up to 100-fold following exercise, returning to baseline levels by 24h following exercise [204]. The anti-inflammatory effects of IL-6 results from the induction of other anti-inflammatory cytokines including IL-1ra and IL-10 [292, 293]. Further evidence of IL-6 on improved glucose homeostasis was demonstrated by IL-6 infusion in T2DM patients, resulting in a decreased plasma insulin response when compared to saline infusion [294]. IL-6 infusion has also been found to increase the glucose infusion rate and glucose oxidation following assessment via a hyperinsulinemic euglycaemic clamp [295]. These studies suggest that IL-6 has insulin sensitising properties and enhances glucose clearance in both healthy and type 2 diabetic individuals. The fact that IL-6 is released following exercise and is dependent upon exercise intensity and duration, highlights the potential impact exercise has on mediating anti-inflammatory effects through the IL-6-induced release of anti-inflammatory cytokine such as IL-10, which is also produced from Tregs [296]. Thus, the pleiotropic role of IL-6 is dependent upon its source, where obesity-induced IL-6 from adipose tissue has pro-inflammatory influence [225], whilst muscle-derived IL-6 is of beneficial effect [297].

The impact of exercise on other inflammatory markers related to T2DM such as adiponectin, MMPs, OPN and Tregs is not yet clear. Together with the obesity-induced reduction in anti-inflammatory markers, current evidence suggests that adiponectin and Tregs are decreased whilst MMPs and OPN are increased in association with obesity and T2DM. Limited evidence is available on the role of exercise training on these markers and its association with glucose control. This part of the review looks to summarize the recent findings of these relevant markers (Table 3.1). A schematic of the relationship between exercise training, inflammation and T2DM can be seen in Figure 1.
Figure 3.1. A schematic of the relationship between exercise training, inflammation and type 2 diabetes.

Adiponectin
Whilst acute exercise has not been found to directly affect adiponectin [298], evidence on exercise in regulating adiponectin levels was previously demonstrated in short- and long-term training studies where as few as three days of exercise, and up to 12 weeks of training, resulted in increased circulating adiponectin in overweight or obese individuals [299-302]. The increase in adiponectin was associated with improved insulin sensitivity in obese individuals further supporting the role adiponectin has on glucose homeostasis as per previous findings [299, 301]. Racil and colleagues suggested that exercise intensity was associated with induction of adiponectin production such that high-intensity intermittent exercise increased adiponectin concentration to a greater degree than moderate intensity exercise when compared to a control group. However, there were no differences of adiponectin between high-intensity and moderate-intensity exercise [301].

Matrix metalloproteinase
Matrix metalloproteinases were initially found to associate with cardiovascular disease and atherosclerosis. However, growing evidence have revealed an association between increased MMPs and T2DM [21-23]. Kadoglou et.al. previously investigated the effect of exercise training for 16 weeks on plasma MMP2 and MMP9 in individuals with T2DM [235]. Although no significant changes were found in MMP2 levels, exercise training significantly reduced MMP9 concentrations, which was also associated with reductions in CRP and HbA1c [235]. These findings demonstrated a link between exercise-mediated improvements in MMP in type
2 diabetics. Kadoglou et al. also investigated the impact of exercise training for 12 weeks in atherosclerotic mice with diabetes [303]. Exercise was beneficial as seen from reduced size of atherosclerotic lesions in mice, which was also associated with reduced concentrations of MMP2, MMP3, MMP9, IL-6 and reduced glucose AUC following an OGTT, further strengthening the impact exercise training has on dampening the inflammatory milieu and glucose tolerance seen with in diabetes and atherosclerosis.

Osteopontin
You et al. found that following 8 weeks of aerobic treadmill exercise in obese females, serum OPN levels were significantly reduced in the morning following the last training session [304]. Despite this positive finding, the effect of exercise remains inconclusive as a number of previous studies did not find any changes in OPN levels [305-307]. A 12-week home-based exercise program in colorectal cancer patients with normal BMI failed to report any significant changes in OPN level [306]. A combination of aerobic and resistance exercise for 12 weeks in overweight/obese middle-aged men with impaired glucose tolerance also failed to show any improvements in OPN concentration [307]. Surprisingly, long term (12 months) training completed in healthy postmenopausal women, consisting of regular (5 days per week) moderate-intensity exercise, did not result in any changes in OPN either [305]. Despite the lack of improvements here, it may be plausible that the reduction in OPN following 8 weeks of treadmill exercise may be associated with weight loss [208], although weight loss through bariatric surgery has been shown to increase serum OPN in morbidly obese individuals [308]. Altogether, these studies highlight mixed findings of OPN following exercise training and is not consistently reduced following exercise training.

Regulatory T cells and IL-10
An understanding of the influence of Tregs on inflammation in T2DM following exercise is still in its infancy as a limited number of studies have been conducted. It is evident in healthy individuals that an acute swimming, increased Tregs number/proportions were found in the blood of elite adolescent swimmers with and without rhinitis/asthma [309]. More relevantly, 12 weeks of Tai-Chi Chuan exercise (meditation type movement) for 60 minutes, increased Tregs numbers in the blood of middle-aged volunteers, and this was associated with increases in plasma levels of TGF-β and IL-10 [310]. This finding is of significance as the increase in TGF-β and IL-10 has been found to drive further production of Tregs which leads to greater suppressive function [267]. Further work found that 12 weeks of Tai Chi Chuan training also increased the number of Tregs in type 2 diabetic patients when compared to a control group [311]. The impact of exercise on Treg numbers in T2DM and its therapeutic potential was further reinforced by a reduction in HbA1c post-training, although the significance and
strength of this association was not assessed [311]. Despite the previous improvement in the number of Tregs reported following Tai Chi Chuan, T2DM individuals who had completed 3 months of endurance exercise training (45 minutes cycling at lactate threshold) did not find increased Treg numbers. Rather, exercise training resulted in a slight decrease which was close to reaching statistical significance [312].

The production of Tregs may also be regulated by exercise intensity as demonstrated from increased Treg cell proportion and production of IL-10 in mice following 6 weeks of high-intensity exercise, but not moderate-intensity [26]. Recent work by Minuzzi et.al. has suggested that the benefit of regular exercise is reflected in the anti-inflammatory status seen following long-term training in masters athletes, who displayed greater levels of IL-10 compared to an age-matched controls, both before and after exercise [144]. Indeed, the anti-inflammatory effect following six months of aerobic exercise training in T2DM patients was effective as seen from increased IL-10 production, which was accompanied with a reduction in CRP as well as improved glucose control and reduced insulin resistance [29]. Similar anti-inflammatory changes were found following 12 months of exercise training where combined aerobic and resistance exercise was demonstrated as the most effective training type resulting in an increase in IL-10 together with reduced CRP, IL-1β and TNFα [44].

### 3.4 Conclusions

The evidence in this review have demonstrated that exercise has been shown to have a mixed effect on the inflammatory response following acute exercise and exercise training. Although acutely, an increased inflammatory response is seen following exercise, long term training generally induces a beneficial adaptation to exercise leading to the reduction of a pro-inflammatory markers together with an increase in anti-inflammatory markers associated with T2DM. Indeed, further work is warranted to investigate the impact the mode, duration and intensity of exercise has on modulating inflammation as well as its relationship with glycaemic control. Although most of the exercise-induced interventions have found positive
improvements, a number of studies have also showed no differences in outcome measures related to inflammation and T2DM. This highlights the gap in the literature where the mechanisms and the understanding in the relationship of chronic inflammation and T2DM is still in its infancy.

In conclusion, chronic low-grade inflammation is a consequence of obesity-induced infiltration of adipose tissue macrophages which increases the concentration of pro-inflammatory markers associated with obesity and T2DM. Fortunately, regular exercise is not only crucial in the management of blood glucose but emerging research has focused on the role of exercise on reducing the risk of developing T2DM by assessing the relationship between markers of chronic inflammation associated with T2DM. In addition to the reduction in traditional inflammatory markers elevated in T2DM such as CRP and IL-6, evidence suggests that regular exercise training is also beneficial in increasing regulatory T cells which are crucial in the production of IL-10 as well as modulating other inflammatory components of the immune system such as matrixmetalloproteinases and osteopontin which have been previously implicated with cardiovascular disease, atherosclerosis and T2DM. This thesis attempts to further investigate the role of exercise on glycaemic control and the subsequent inflammatory response following acute exercise and a short-term training period.

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<th>Marker</th>
<th>Roles in obesity/T2D</th>
<th>Role in exercise</th>
<th>Role in glucose control</th>
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<tr>
<td>IL-1β</td>
<td>↑ IL-1β = ↑ risk of T2DM [11, 13, 46].</td>
<td>↑ IL-1β following acute exercise in highly trained and untrained post-exercise [313].</td>
<td>High glucose induces production of IL-1β in β-cells [230] Blocking of IL-1β reduces hyperglycaemia [209]</td>
</tr>
<tr>
<td>IL-6</td>
<td>↑ IL-6 correlated with reduced IS [217] and insulin resistance [218].</td>
<td>Acute exercise ↑ IL-6 [47, 204, 276, 313].</td>
<td>↑ IL-6 under high glucose conditions in vitro [213, 315]</td>
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<td>Marker</td>
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<tr>
<td>MMP2</td>
<td>↑ MMP2 in T2DM patients vs healthy[21, 317].</td>
<td>↓ MMP2 following 12 weeks exercise training [303]. ↑MMP2 following 10 days of exercise [318].</td>
<td>High glucose exposure in endothelial cells ↑MMP2 [238].</td>
</tr>
<tr>
<td>MMP3</td>
<td>↑ MMP3 in T2DM patients [23, 319].</td>
<td>↓ MMP3 following exercise training [303].</td>
<td>↑ MMP3 inhibits glucose uptake and promotes TNFα [199].</td>
</tr>
<tr>
<td>TNFα</td>
<td>↑ TNFα associated with insulin resistance [14, 15, 222] and ↓IS [223]. TNFα higher in IGT compare to control subjects [320].</td>
<td>↑ TNFα with acute exercise [313]. Exercise training ↓ TNFα and ↑ insulin sensitivity [320].</td>
<td>↑ TNFα in impaired glucose tolerance compared to normal glucose tolerance [214].</td>
</tr>
<tr>
<td>CRP</td>
<td>↑ CRP in individuals with impaired glucose tolerance and T2DM [19, 46, 197, 217].</td>
<td>Exercise training ↓ CRP [29, 57].</td>
<td>Positively correlated with insulin [10, 57]. Inversely associated with insulin responsiveness [217].</td>
</tr>
</tbody>
</table>
| Tregs | ↓ Tregs in obesity and T2DM [263-265].  
↓ Tregs = ↑ IL-10 production [262]. | ↑ Tregs after acute exercise [309].  
↑ with training [310, 311]. |  
| IL-10 | ↓ IL-10 = ↑ risk of T2DM [203, 302].  
Impaired glucose tolerance = ↓ IL-10 [17].  
Treatment of IL-10 = ↑ insulin signalling + ↑ glucose uptake + ↓ insulin resistance [321]. | ↑ IL-10 post-exercise, greater in high-intensity exercise compared to moderate-intensity [322]. | ↓ IL-10 associated with high glucose & HbA1c [203]. |

**Table 3.1.** The relationship of pro-inflammatory and anti-inflammatory markers to T2DM, exercise and glucose control
Chapter 4  

Effect of exercise on acute postprandial glucose concentrations and interleukin-6 responses in sedentary and overweight males


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4.1 Abstract

This study examined the effect of two forms of exercise on glucose tolerance and concurrent changes in markers associated with the interleukin-6 (IL-6) pathways. Fifteen sedentary, overweight males (29.0±3.1 kg/m²) completed two separate, 3-day trials in randomised and counterbalanced order. An oral glucose tolerance test (OGTT; 75g) was performed at the same time on each successive day of the trial. Day two of each trial, consisted of a single 30-min workload-matched bout of either high-intensity interval exercise (HIIE; alternating 100% and 50% of VO₂peak) or continuous moderate intensity exercise (CME; 60% VO₂peak) completed 1h prior to the OGTT. Venous blood samples were collected pre-, immediately post-, 1h post- and 25h post-exercise for measurement of insulin, C-peptide, IL-6 and the soluble IL-6 receptors (sIL-6R; sgp130). Glucose area under the curve (AUC) were calculated from capillary blood samples collected throughout the OGTT. Exercise resulted in a modest (4.4%; p = 0.003) decrease in the glucose AUC when compared to the pre-exercise AUC; however, no differences were observed between exercise conditions (p = 0.65). IL-6 was elevated immediately and 1h post-exercise, whilst sgp130 and sIL-6R concentrations were reduced immediately post-exercise. In summary, exercise was effective in reducing glucose AUC which was attributed to improvements between 60 and 120 min of the OGTT, was in parallel with an increased ratio of IL-6 to sIL-6R, which accords with an increased activation via the ‘classical’ IL-6 signalling pathway. Our findings suggest acute HIIE did not improve glycaemic response when compared to CME.

**Trial registration:** ACTRN12613001086752

**Key words:** Obesity, glucose regulation, interval training, area under the curve, high-intensity
4.2 Introduction

Regular exercise is beneficial in the management of blood glucose and reducing the risk of type 2 diabetes [27]. The exercising muscle stimulates glucose transporter-4 (GLUT4) translocation and the associated increase in glucose uptake capacity during exercise is dependent upon both the intensity [323-325] and duration of the activity [326, 327]. In combination with increased blood glucose delivery and intramuscular metabolic flux [328], the increased glucose transport capacity resulting from GLUT4 translocation can result in a 7-fold (light exercise; ~63% max heart rate, HR_max), ~10-fold (moderate-heavy exercise;~80-90% HR_max) and ~16-fold (~85% W_max) increase in leg glucose uptake with increasing exercise intensity [277, 325]. Increased (above baseline) glucose uptake can persist for up to 48 h after exercise [329]. However, despite this localised improvement in glucose uptake, systemic postprandial glucose (PPG) tolerance remains either unchanged [330-333], increases [334-339] or decreases [340-342] in the hours following an acute bout of moderate- and high-intensity exercise. The disparity between peripheral and systemic glucose concentrations may be due to an increased rate of hepatic glucose release, which continues for approximately 30 min after cycling in overweight/obese individuals with normal glucose tolerance [343] as well as a concomitant decrease in insulin sensitivity in tissues other than the contracting musculature [328, 333, 343].

Interleukin-6 (IL-6) has been implicated in controlling glycaemia and the concentration of circulating IL-6 in response to exercise has also been shown to increase in an intensity-dependent manner [47, 204, 344]. Acutely, IL-6 increases glucose disposal [295, 345] in young healthy males, although not in males with type 2 diabetes mellitus (T2DM) [346]. While chronically elevated resting IL-6 concentration has been identified as a risk factor for developing T2DM [46, 347] and is associated with poorer glycaemic control in individuals with existing T2DM [348]. This equivocal relationship between IL-6 and glucose regulation may arise as a consequence of the two distinct IL-6 signalling pathways. ‘Classical signalling’ occurs following binding of IL-6 to the membrane bound IL-6 receptor (IL-6R) and associated glycoprotein 130 (gp130), forming an IL-6—IL-6R—gp130 complex [349]. ‘Trans-signalling’ occurs when IL-6 binds to a soluble form of IL-6R (sIL-6R), and where this IL-6—sIL-6R complex now directly binds gp130 at the plasma membrane to form the IL-6—sIL-6R—gp130 complex [349, 350]. Since gp130 is ubiquitously expressed, cells which do not express the membrane bound IL-6R may now become IL-6 responsive via this trans-signalling pathway. It has been postulated that trans-signalling has detrimental effects on insulin sensitivity by mediating the pro-inflammatory effects of IL-6, while the classical signalling pathway promotes glucose uptake [350]. Trans-signalling may be inhibited via binding of
soluble gp130 (sgp130) to the circulating IL-6—IL-6R complex; importantly, the sgp130 does not bind either IL-6 or IL-6R alone and does not therefore affect the classical pathway [349].

Whether the inconsistent effect of acute exercise on PPG response [330-342] and more specifically, the inconsistent findings regarding the role of exercise-intensity on PPG response [186, 187, 351-353], is in part explained by the different responses in sIL-6R and sgp130 to exercise, is less clear. Therefore, the purpose of the current study was to examine the effect of exercise on IL-6, sIL-6R and sgp130 concentrations in sedentary and overweight males, and assess glucose tolerance during a subsequent glucose challenge. We hypothesised that (i) acute high-intensity intermittent exercise (HIIE) would improve PPG (glucose area under the curve) to a greater degree than a work- and duration-matched continuous exercise bout, and this will persist for up to 24 hours following exercise; (ii) Acute exercise will increase IL-6, and decrease sIL-6R and sgp130 concentrations, wherein these effects will be amplified following HIIE versus a work- and duration-matched continuous exercise bout.
4.3 Methods

4.3.1 Study Design

The study was completed using a randomized cross-over design. All participants attended the Exercise Physiology labs at Murdoch University for a total of seven visits (introductory session; two experimental sessions comprising three visits each). The introductory session (visit 1) involved the consent process, a familiarization session and assessment of fitness (peak oxygen uptake, $\dot{V}O_{2peak}$; maximal aerobic power assessment). The two experimental phases comprised three study days each (Day-1, Day-2, Day-3), and were completed using a randomized cross-over design, with order randomization and allocation conducted by a researcher not involved in the delivery of the intervention (TJF). Participants were informed of the possible risks associated with the study before giving written informed consent. Ethics approval was granted by Human Research Ethics Committee at Murdoch University (Project number: 2013/121). This trial was prospectively registered with Australian New Zealand Clinical Trials Registry: ACTRN12615000613505.

4.3.2 Participants

Fifteen sedentary ($\leq$2 bouts of low-to-moderate intensity exercise per week) and overweight (BMI $>25$ kg/m$^2$) males were recruited from the local community via advertisements posted on public noticeboards. Participants were deemed eligible if they were aged between 18 and 44 years old, had not been previously diagnosed with T2DM (fasting plasma glucose $<7$ mmol/L) and had not smoked within the previous 6 months. Participants were excluded if they were taking anti-inflammatory medication, glucose controlling drugs (i.e. metformin), had uncontrolled hypertension, and had either an acute or chronic inflammatory disease/infection. Participants were pre-screened for exercise using the Exercise Science and Sport Science Australia (ESSA) medical screening tool.
4.3.3 Introductory Session

On arrival, participants were fully informed of all study procedures and written informed consent was obtained. Body height, body mass and waist circumference were determined. Body composition was measured using the Dual Energy X-ray Absorptiometry (DXA; Hologic Discovery QDR series, Hologic Inc., Bedford, MA, USA). Thereafter, participants completed an incremental cycling test (50W; with 25W increments every 2 min) until volitional exhaustion on an electronically braked Velotron cycle ergometer (RacerMate, Seattle, WA, USA) for measures of VO2peak. During the incremental cycling test, expired ventilation was collected as 15-second mean values using Parvomedics TrueOne metabolic cart (Parvomedics, Sandy, UT, USA) and heart rate (Polar T31, Kempele, Finland) was collected at 1 Hz. VO2peak was determined as the highest 30s average VO2 from the final stage of the incremental test.

Heart rate and Borg’s [354] rating of perceived exertion (RPE) was collected throughout the maximal graded exercise test. This session was completed at least three days prior to the first experimental phase.

4.3.4 Experimental Phases

On Day-1 of the experimental phase, participants reported to the laboratory in a fasted state at ~8am during which an oral glucose tolerance test (OGTT; 75g Carbotest, ThermoFisher, Australia) was completed. Capillary blood samples were obtained via finger stick prior to the OGTT, and at 15, 30, 60, 90 and 120 min following ingestion of the solution for measurement of glucose (Hemocue Glucose 201 RT glucose; Hemocue AB, Ängelholm, Sweden). The Hemocue glucose analyser uses a dual wavelength photometer that measures glucose following a modified glucose dehydrogenase reaction and has been validated against the Yellow Springs Instrument glucose oxidase analyser [355]. Participants were then provided with a standardized breakfast (Up and Go, Sanitarium, Australia; two muesli bars, Kellogg’s, Australia) before leaving the laboratory. Food intake for the remaining portion of the day was at the participants’ discretion and was recorded in a food diary and repeated in the subsequent trial.
Twenty-four hours after the initial OGTT, and following an overnight fast, participants arrived at the laboratory for their second visit in which they provided a venous blood sample (8ml) from the antecubital vein collected into EDTA and SST tubes (Becton Dickinson, NJ, USA). Participants then, completed a warm up at 75W before completing the assigned exercise condition. Immediately upon the cessation of exercise, a second venous blood sample was collected, followed by an hour of recovery where participants were advised to relax and remain seated throughout the recovery period prior. A third venous blood sample was collected after the recovery period prior to the completion of an OGTT using the same methodology as the initial OGTT (Day-1) and at the same time of day. Participants were then provided with the standardized breakfast before leaving the laboratory.

Day-3 comprised the same design as Day-1, with participants reporting to the laboratory in a fasted state with the final venous blood sample collected prior to the final OGTT at the same time as the previous days. A schematic of the timeline can be seen in Figure 4.1.

Figure 4.1. Diagram of study design.

4.3.5 Exercise Conditions (Day-2)

Each of the two experimental phases followed an identical three-day design, with the exception being the exercise condition performed on Day-2. Day-2 comprised either a continuous moderate intensity exercise bout (CME; 30 min at 60% of VO2peak), or a high intensity intermittent exercise (HIIE) bout. Participants completed the desired workload based on power output derived from the VO2peak test. The Velotron bike is electromagnetically braked and the wattage was pre-set according to the protocol for each condition. The HIIE bout comprised six efforts at high- (1 min at 100% VO2peak) and low- (4 min at 50% VO2peak) intensity which lasted 30 min in duration. Participants were advised to maintain a cadence between 80-110 revolutions per min (rpm) during the intense bouts and to maintain a comfortable cadence during the recovery period. This exercise protocol was based on a previously published protocol [356]. Both exercise conditions preceded with a standardized warm-up at 75W for five min. Heart rate and RPE were recorded throughout each exercise trial.
4.3.6 Blood Analysis

Serum samples (SST; 5mL) were allowed to clot for at least 30 min at room temperature, before subsequently being centrifuged at 1,300g for 15-min. Plasma samples (EDTA; 6mL) were spun immediately at 1,300g for 15-min. The supernatant was then immediately aliquoted into triplicates and stored at -80°C. Samples were batch analysed for plasma glucose using a commercial kit (GAHK20, Sigma-Aldrich, MO, USA); serum insulin and C-peptide (Bio-Plex Pro Human Diabetes Assay, CA, USA; Inter-assay coefficient of variation, insulin: 5.0%, C-peptide: 4.0%; Limit of detection, insulin: 1.0pg/ml, C-peptide: 14.5pg/ml); interleukin-6 receptor (sIL-6R; Inter-assay coefficient of variation: 3.2%; Limit of detection: 1.5pg/ml) and glycoprotein 130 (sgp130; Bio-Plex Pro Human TH17 Assay, CA, USA; Inter-assay coefficient of variation: 5.9%; Limit of detection: 16.9pg/ml) using the Bio-plex MAGPIX multiplex reader (Bio-Rad, CA, USA). IL-6 was measured in serum using a high-sensitivity enzyme-linked immunosorbent assay (ELISA; R&D Systems Human IL-6 Quantikine HS-ELISA; MN, USA; Inter-assay coefficient of variation: 7.8%; Limit of detection: 1.77pg/ml).

Analyses of the standard curve for sgp130 and IL-6R used 5PL (Five Parameter Logistic) equation which optimises the fit of the standard curve by default. The output provides an assessment of the ‘goodness’ of the fit providing statistical parameters such as ‘fit probability’ (where 1 is a perfect fit and 0 has no fit) and ‘residual variance’ (where a small residual variance indicates a good fit). The fit probability and residual variance for sgp130 and IL-6R were: 0.829, 0.295 and 0.889, 0.210, respectively, which is similar to the manufacturer’s data. These statistical parameters should also be interpreted with slight caution as it is a probability outcome measuring the quality of fit, rather than R-squared which is not a sensitive metric for assessing the quality of a curve fit.
4.3.7 Data Analyses

Data are presented as mean ± SD, unless otherwise stated. Glucose concentrations from the oral glucose tolerance test are presented using the total glucose area under the curve (AUC) using the trapezoidal rule (Graphpad, PRISM), and as comparisons in raw glucose values using 95% confidence limits between corresponding days [357]. Separate bivariate regression analysis was conducted to explore the associations between (i) glucose AUC on Day-2, with the AUC (time-points: pre-exercise, immediately post-exercise, 1h post-exercise) of IL-6, sIL-6R, sgp130; and (ii) glucose AUC on Day-3 with the concentrations of IL-6, sIL-6R, sgp130 on Day-3 (25h concentration). Data management and statistical analyses were performed using IBM SPSS version 22.0 software (IBM Corp, Armonk, NY). Treatment effects were estimated using separate, random-intercept linear mixed models (LMM) for each outcome variable (blood analytes; heart rate; RPE; glucose AUC). Condition (CME, HIIE) and time (i.e., pre-exercise, post-exercise, 1h post-exercise and 25h post-exercise; or, within exercise time-points) were modelled as fixed effects. The primary hypothesis of interest was the time by group interaction with main effects of condition and time examined with pairwise comparisons of the estimated marginal means and reported accordingly. To assess magnitude of change, Cohen’s $d$ effect sizes were calculated and classified as: small effect (>0.2); moderate effect (>0.5), and; large effect (>0.8) [358]. Significance was accepted at $p \leq 0.05$. 

__________________________________________
Participant Characteristics
__________________________________________
<table>
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<td>Height (m)</td>
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<tr>
<td>Weight (kg)</td>
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<td>BMI (kg/m²)</td>
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<td>FBG (mmol/L)</td>
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<tr>
<td>WC (cm)</td>
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</tr>
<tr>
<td>Total fat mass (kg)</td>
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</tr>
<tr>
<td>Fat (%)</td>
<td>27.5 (4.2)</td>
</tr>
<tr>
<td>V̇O₂ (ml/kg/min)</td>
<td>35.7 (5.1)</td>
</tr>
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</table>

Table 4.1 Descriptive characteristics of the participants. All data are presented as mean (SD). FBG, Fasting blood glucose; WC, waist circumference; V̇O₂, peak V̇O₂ achieved.
4.4 Results

Descriptive data of participants are displayed in Table 4.1. All participants completed the two experimental phases. One participant completed only 5 sprints during the HIIE bout, whilst one other participant completed all six sprints in the HIIE bout, but did not complete the last four min (low-intensity portion of the protocol) of the bout. All data were included in the analyses.

Mean RPE (mean±SD) was higher (p<0.01) during exercise for HIIE (16 ± 2) compared to CME (14 ± 2; Figure 4.2). Accordingly, the interaction of trial by time revealed greater perceived effort during HIIE than CME from at the time point corresponding from bout-3 to bout-6 (Figure 4.2). Mean heart rate during exercise was higher during HIIE when compared to CME; 147 ± 8 beats per min and 139 ± 11 beats per minute, respectively. There was no main effect for HR between exercise conditions. Figure 4.2; p=0.080). Energy expenditure (mean±SD) following CME (268 ± 31 Cal) was not different to HIIE (260 ± 10 Cal; p=0.167).

**Figure 4.2.** (Top). Mean (± SD) Ratings of perceived exertion after completing high-intensity intermittent exercise (HIIE, filled square) and continuous moderate-intensity (CME, open circle) using Borg’s scale. a, significantly different from CME (P < 0.01). (Bottom). Mean (± SD) Heart rate response during high-intensity intermittent exercise (HIIE) compared to continuous moderate-intensity (CME). No significant differences between trials p=0.112.
4.4.1 Glucose Responses

The plasma glucose AUC are presented in Figure 4.3. A main effect of time (p<0.001) such that plasma glucose AUC was significantly lower on Day-2 following exercise when compared to Day-1 (mean difference [95% CI] in glucose: -0.36 [-0.65, -0.08] mmol/L; ES: 0.755) and Day-3 (-0.56 [-0.79, -0.34] mmol/L; ES: 1.078) was observed. However, no other differences, interaction (condition x day; p=0.302) or main effect of condition (i.e. between exercise conditions; p=0.912) were observed.

In accordance with the AUC data, there were no differences between conditions on Day-2 (OGTT conducted 1 h post exercise) or Day-3 (25h post exercise) (Figure 4.3). When the glycaemic response was explored between Day-2 and Day-1, exercise (HIIE and CME combined; Figure 4.4) was shown to reduce the plasma glucose concentrations at 60 min, 90 min and 120 min of the OGTT. The AUC on Day-3 showed a significant increase at 15, 30, 60 and 90 min of the OGTT, while no differences between Day-3 and Day-1 were identified (data not shown).

**Figure 4.3.** Box and whiskers (95% CI) plot for plasma glucose area under the curve (AUC) on Day-1 (baseline), Day-2 (exercise) and Day-3. A main effect of time was present; p=0.000.
Figure 4.4. Mean differences (with 95% CI) in plasma glucose concentration between A: HIIE and CME on Day-2; B: HIIE and CME on Day-3; C: Day-2 and Day-1; D: Day-3 and Day-2. Differences were calculated from blood glucose concentrations for each participant at each time-point of the OGTT from one study day and subtracted from the corresponding time-point on the comparison study-day. Significant differences are indicated where 95% confidence limits to not cross the zero band.

4.4.2 Insulin, C-peptide and Inflammatory Markers

Data from all participants were available for analysis insulin and C-peptide, whilst data from only ten participants were available for the analysis of the inflammatory marker panel. A main effect time (C-peptide: p=0.001; insulin: p=0.007) was observed. However, no other differences, interaction (condition x day; C-peptide: p=0.296; insulin: p=0.154) or main effect of condition (C-peptide: p=0.283; insulin: p=0.443) were observed. When compared to pre-exercise concentrations, both insulin and C-peptide concentrations demonstrated similar trends with concentrations significantly elevated immediately post-exercise and lower 1 h and 25 h after exercise (Figure 4.5).
There was a main effect of time on IL-6 (p=0.001) concentrations. When compared to immediately post-exercise and 1 h post-exercise concentrations (Figure 4.6), IL-6 was lower at pre-exercise (p=0.033, ES:-0.461; p=0.011, ES:-0.607, respectively) and 25 h post-exercise (p=0.002, ES:-0.806; p<0.001, ES:-1.053, respectively). A main effect for condition was not present, nor was a time by condition interaction. A main effect of time was present for both sgp130 (p=0.006) and sIL-6R (p=0.010), with significantly lower concentrations in both sgp130 and sIL-6R immediately post-exercise when compared to 1 h post-exercise (p=0.007, ES:-0.558; p=0.019, ES:-1.012, respectively) and 25 h post-exercise (p=0.001, ES:-0.696; p<0.001, ES:-0.905, respectively). No significant differences were observed in the IL-6/sIL-6R complex concentration between the two trials (p=0.287), although a main effect of time was present (p<0.001, Figure 4.6). When compared to immediately post-exercise and 1 h post-exercise, IL-6/sIL-6R complex concentration was lower at pre-exercise (p=0.005, ES:-0.721; p<0.001, ES:-0.805, respectively) and 25 h post-exercise (p<0.001, ES:-0.806; p<0.001, ES:-1.242, respectively). There were no significant correlations obtained during either the HIIE or CME conditions between the glucose-AUC and sGP130 (All p≥0.329) or sIL-6R (All p≥0.222). However, the Day-2 (averaged across pre-exercise, immediately post-exercise and 1 hr post exercise) IL-6 concentration demonstrated a positive association with glucose AUC (CME: r=0.627, p=0.053; HIIE: r=0.658, p=0.039), and this trend (non-significant) was also observed between glucose-AUC and the ratio of IL-6/sIL-6R on Day-2 in the CME condition (r=0.622, p=0.055) but not the HIIE condition (r=0.400, p=0.252).
Figure 4.5. Box and whiskers (95% CI) plot for insulin (Top) and c-peptide (Bottom) measured pre-exercise (baseline), immediately post-exercise (IPE), 1h post-exercise and 25h post-exercise. a) compared to pre-exercise, p<0.05; b) compared to post-exercise, p<0.01; c) compared to post-exercise, p<0.05. Open bars, CME; hatched bars, HIIE.
Figure 4.6. Box and whiskers (95% CI) plot for: A: IL-6; B: sIL-6R; C: IL-6/sIL-6R complex, and D: sgp130; measured in 10 participants pre-exercise (baseline), immediately post-exercise (IPE), 1h post-exercise and 25h post-exercise. a) compared to IPE and 1h post-exercise, p<0.05; b) compared to IPE and 1h post-exercise, p<0.01; c) compared to 1h post-exercise and 24h post-exercise, p≤0.01. Open bars, CME; hatched bars, HIIE.
4.5 Discussion

This study sought to determine the effects of acute HIIE and CME on blood glucose responses during an OGTT performed 1 h and 25 h post exercise in sedentary, overweight/obese men. The main findings were: i) glucose AUC was significantly lower during the OGTT performed 1 h post exercise (Day-2) than compared to baseline (Day-1) or 25 hours after exercise cessation (Day-3); ii) This reduction in glucose AUC appeared to result from a significant reduction in glucose concentrations between 60-120 min of the OGTT when compared to baseline (Day-1); iii) There were no differences in the glucose responses between HIIE or CME conditions. To assess the role of IL-6 in moderating the effects of exercise on glucose tolerance, IL-6, sIL-6R and sgp130 concentrations were also examined. The concentration of IL-6 increased immediately post-exercise and 1 h post-exercise, while the concentration of both sIL-6R and sgp130 decreased immediately post-exercise. Consequently, the ratio of IL-6 to sIL-6R was elevated immediately post-exercise and 1 h post-exercise which is expected to result in a proportionate increased activation of the classical IL-6 signalling pathway; while the concurrent changes in sIL-6R and sgp130 concentrations expected to result in the net activation of the IL-6 trans-signalling pathway remained largely unaffected.

The finding that exercise reduced the subsequent glucose AUC during an OGTT in this study is in accordance with our stated hypothesis and findings of some [334-339] but not all [330-333, 340-342] previous studies. These differences are likely attributed to the timing of the OGTT relative to the cessation of exercise. Indeed, higher glucose AUCs (i.e., worse) have been observed when the OGTT is performed within 30 min of exercise cessation [340-343] compared to; i) 60 min later [339]; ii) over the subsequent 24 h period (continuous glucose monitoring; [334]); iii) or 24 h after cessation of exercise [340, 342]. The underlying mechanism explaining this disparity may reside in the increased net hepatic glucose release occurring during exercise and which continues for approximately 30 min after cycling in overweight/obese individuals with normal glucose tolerance [343]. Since exercise in this study was initiated 60 min post-exercise, it is unlikely that hepatic glucose release was still elevated. The observed reduction in glucose AUC between 60 to 120 min in this study is consistent with findings of Rynders et al. [339]. Of relevance to our findings, Knudsen et al. [343] observed peak glucose disappearance ~90-100 min into the OGTT of individuals who were overweight/obese but of normal glucose tolerance, both at rest and post-exercise. Although glucose appearance/disappearance was not measured in the present study, the improved glycaemic response is likely the result of the exercise-stimulated GLUT4 translocation to the sarcolemmal membrane [323-325, 359].
In the current study, the glucose AUC during the OGTT on Day-3 was similar to Day-1 and significantly higher than the glucose AUC obtained 1 h post-exercise; indicating that the beneficial effect of exercise on glucose tolerance had disappeared 24 h post exercise. This finding is in contrast to the stated hypothesis and the findings of Bonen, Ball-Burnett [337] and King, Baldus [340] whom observed significantly improved glucose AUCs 24 h post exercise. However, differences in findings may partly be explained by the age of participants and differences in the exclusion criteria between studies. The current study excluded individuals who were physically active or had a BMI ≤ 25 kg.m$^{-2}$ and had a mean participant-age of 28 years old. King, Baldus [340] recruited moderately trained and lean individuals, whilst Bonen et al. (1988) recruited middle-aged (40 to 55 years old) inactive individuals but without exclusion based on BMI (men: 27.3 ± 0.7; women: 23.6 ± 1.5).

The responses in insulin and C-peptide following the exercise protocols are in agreement with previous work [360-362]. Specifically, low-moderate intensity exercise results in a well characterized decrease in insulin concentration, however supra-maximal (or very high-intensity) exercise has been associated with a transient (min-hours) increase in insulin and C-peptide which may be explained by a 14- to 18-fold increase in catecholamines [339, 361]. Indeed, we hypothesized that the increase in inflammatory markers in individuals unaccustomed to exercise would partly negate the benefits of exercise on the glucose AUC. However, this appeared not to be the case given i) the 1 h post-exercise AUC was improved; ii) there were no significant associations between any of the measured inflammatory markers and glucose AUCs on Day-2 or Day-3. It is important to note however, that the exercise-induced decrease in the glucose AUC (Day-2) in the current study, while significant, was smaller than anticipated. It is possible, that a concomitant decrease in insulin sensitivity in tissues other than the contracting musculature [328, 333, 343] contributed to the relatively small improvement in the systemic glucose AUC post-exercise.

Although resting IL-6 is associated with increased adiposity [215] and an increase in risk of T2DM [46], the transient release of IL-6 from muscle during acute exercise can promote muscle glucose uptake and insulin action [277, 295, 363]. The increase in circulating IL-6 concentration following exercise is dependent on the mode, duration and intensity of exercise [47, 276, 364-366]. The anti-inflammatory effect of IL-6 is believed to be associated with the improved glucose control [346, 349, 350], and is associated with the release of IL-10 [291]. In accordance with the findings from this study, acute HIIE has been shown to increase IL-6 concentration [364, 367]. Although IL-10 was not measured in the current study, previous work reported an increase in both IL-6 and IL-10 post HIIE [368], with the increase in IL-6 occurring 45 min prior to the increase in IL-10. It is important to note that the current study
did not see a difference between exercise conditions, which is in contrast to the findings of Leggate, Nowell [364], who observed significantly greater IL-6 concentrations following HIIE compared to work-matched CME in lean and physically active participants. While the participants in the study of Leggate et al. (2010) were different, the exercise protocol of Leggate et al. (2010) was 60 min in duration (HIIE: 10 x 4 min, recovery 2 min) while the current study was only 30 min in duration. Considering IL-6 has previously been postulated to act as a signalling molecule indicating muscle glycogen levels (greater glycogen depletion leading to greater IL-6 release) [365], it is possible the 60 min exercise duration adopted by Leggate et al. (2010) resulted in a greater total depletion of muscle glycogen which was sufficient to see a difference between the two exercise protocols.

Interestingly, both sgp130 and sIL-6R were lowest immediately post-exercise. When IL-6 is expressed relative to sIL-6R (IL-6/sIL-6R; Figure 4.5), the post-exercise increase is enhanced, which is in accordance with previous findings [364]. This finding is important since a concomitant increase in IL-6 and sIL-6R is expected to increase activation of the ‘trans-signalling’ pathway, while a reduction in sIL-6R is expected to reduce signalling via this pathway due to reduced extracellular binding capacity of IL-6—IL-6R. The trans-signalling pathway has been implicated in decreasing glucose tolerance in the longer-term and is associated with infiltration of adipose tissue macrophages [350]; correspondingly, sIL-6R is elevated in T2DM [348]. The purported deleterious effects of trans-signalling on glucose regulation, may be ameliorated via the binding of sgp130 to the circulating IL-6—IL-6R complex [349], since this reduces the availability of the complex to bind membrane-associated gp130. It is interesting to note the corresponding time-course of sgp130 and IL-6R in the current study, which indicates the trans-signalling pathway is unlikely to have been altered to any great degree. In contrast, the increase in IL-6 with the concurrent decrease in sIL-6R is consistent with an increased activation of the classical IL-6 signalling pathway which is linked with stimulating increased skeletal muscle glucose uptake [369]. Multiple independent correlations were conducted to explore associations between IL-6 and IL-6 related proteins, and whilst caution is required in the interpretation of multiple repeated correlations on a small sample, it is of note that positive relationships between glucose-AUC and both IL-6 and ratio of IL-6/IL-6R were observed, which would be in accordance with an increased activation of the classical signalling pathway as proposed. Further work in this field would benefit from the
adoption of co-immunoprecipitation methods to explore these interactions in greater detail.

The study herein had a number of strengths including i) matching the workload and duration of the HIIE and the CME conditions; ii) recruiting a clinically relevant population, namely individuals who are inactive and overweight- which has, to our knowledge not previously been conducted in a study of this kind; iii) adopting a randomized cross-over design; iv) each trial included a specific baseline AUC which was conducted 24 hour prior to the testing sessions and included a 25 h post AUC (with all AUCs being conducted at the same time of day). However, there were also some limitations associated with this study. Specifically, the number of blood samples collected by venepuncture were limited, and therefore restricted the information on IL-6 and IL-6 receptors, as well as insulin and glucagon during the OGTT which would have enhanced our interpretation of findings. Additionally, despite IL-6 and its receptors being measured in serum, the study did not measure direct activation of each signalling pathway, and can therefore only postulate on the changes based on the serum markers. While an obese and sedentary population was recruited, inclusion of a broader group of individuals spanning healthy to T2DM would have allowed assessment across clinical and pre-clinical stages. Finally, although glucose tolerance was assessed, it must be stressed that our findings cannot confirm increased glucose disposal/clearance as an isotope tracer technique was not employed to measure hepatic glucose production in the present study.

In conclusion, 30-min of acute exercise reduced the glucose AUC during the OGTT performed one-hour post exercise, although the improvements in glucose AUC were ameliorated 25 hours later. The changes in IL-6, sIL-6R and sgp130 are consistent with increased activation of the ‘classical’ signalling pathway immediately post-exercise and 1 h post-exercise, which is postulated to have contributed to the improvement in post-exercise glucose AUC. Further work is warranted to directly assess the activation of the IL-6 pathways in response to both acute exercise and exercise training in pre-clinical and clinical populations. Additional methods such as isotope-labelled tracer technique should also be employed in order to accurately determine the direct impact of these pathways on glucose disposal across tissues. The understanding of these pathways has clinical implications, particularly with respect to exercise prescription, wherein decisions on frequency, mode, intensity and duration of exercise are made. Finally, the acute HIIE protocol did not confer additional benefits on glucose concentration, or alter either the endocrine or IL-6 and IL-6 related soluble receptors when compared to a work- and duration-matched CME protocol.

Conflicts of interest: The authors have no conflicts of interests to declare.
Chapter 5  Glucose and inflammatory markers following acute exercise in sedentary and overweight males

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The manuscript in this chapter is currently under review, submitted to the Journal of Applied Physiology.
5.1 Abstract

Inflammatory markers associated with adipose-tissue expansion have been implicated in the early progression of metabolic disease. Consequently, Matrix metalloproteinase-2 (MMP-2) and -3 (MMP-3), and osteopontin have garnered recent attention. The purpose of the current study was to assess the circulating concentration of these markers, along with adiponectin and glucose concentrations in response to two different modes of exercise. Fourteen sedentary, overweight males (29.0 ± 3.1 kg/m²) completed two separate, 3-day trials in randomised and counterbalanced order. Oral glucose tolerance tests (OGTT) were performed on each day of the trial. Day two of each trial, consisted of a single 30-min workload-matched bout of either high-intensity interval exercise (HIIE; alternating 100% and 50% of VO2peak) or continuous moderate intensity exercise (CME; 60% VO2peak) completed 1h prior to the OGTT. Continuous glucose and physical activity monitoring occurred throughout each trial, and venous blood samples were collected pre-, 0h post-, 1h post- and 25h post-exercise for measurement of MMP-2, MMP-3, osteopontin and adiponectin. No significant trial-by-time interaction effects were noted for MMP-2, MMP-3, osteopontin or adiponectin. There was a main effect of time (both p<0.01) with exercise transiently increasing MMP-3 and decreasing osteopontin, but both returned to baseline values by 1h post-exercise. Exercise did not improve glucose values over the subsequent 48h. Mean day glucose and area under the curve from the OGTT was higher on Day 2 and 3 compared to day 1 (main effect of time; both p<0.05). Acute exercise improved risk markers of metabolic disease without improvements in glucose control.

Trial Registration: ACTRN12613001086752

Key Words: Obesity, cardiovascular disease, glucose regulation, interval training, high-intensity
5.2 New & Noteworthy

This study demonstrated that regardless of exercise intensity, exercise was effective in inducing changes to MMP-3 and OPN immediately post-exercise. We also assessed continuous glucose concentrations for 72h to assess glycemic control under free-living conditions. Interestingly, no improvements in glucose tolerance were evident over the three-day period. Whereas previous study has shown an improvement in glucose response within 24 h following exercise.
5.3 Introduction

The adipose tissue expansion associated with obesity results in the infiltration of macrophages expressing pro-inflammatory cytokines [241, 370, 371]. The resultant nett increased release of pro-inflammatory cytokines from adipose tissue can impair insulin signalling [9] and lead to microvascular and macrovascular complications [220, 372-374]. These complications underpin the increased rates of morbidity and mortality from cardiovascular disease in individuals with diabetes mellitus [375, 376].

Attention has now focussed on identifying the initiating markers implicated in the shift to the predominantly pro-inflammatory state associated with obesity and obesity-related disease states [25, 377]. In this vein, matrix metalloproteinases (MMPs) appear to play potentially important roles early in obesity-related disease progression. In particular, adipose tissue releases MMP-2 during adipocyte differentiation, which is understood to regulate the vascular extracellular matrix (ECM) remodelling during obesity-induced adipose tissue formation [48, 49]. In contrast, the release of MMP-3 appears to have an inhibitory effect on adipocyte differentiation and therefore hyperplastic adipose expansion [50]. In an in vitro cell culture model utilising human umbilical vein endothelial cells (HUVEC), hyperglycaemia (25 mM) significantly increased concentrations of MMP-2 and reduced concentrations of MMP-3 [238]. This altered pattern in MMPs is further observed in clinical populations, with increased MMP-2 concentrations in obese patients [22]; increased MMP-2 concentrations [21] and decreased MMP-3 concentrations in patients with type 2 diabetes mellitus (T2DM; [23]).

Osteopontin (OPN) has also emerged as a potentially important regulator of inflammation, obesity and diabetes [24]. Whilst OPN appears to play multiple immune-modulating roles, the roles of OPN including the regulation of macrophage movement and accumulation [51] and stimulating expression of MMP-2 [52] are of particular interest herein. Accumulation of macrophages, particularly classically activated macrophages in adipose tissue acts as a source for pro-inflammatory cytokines. These cytokines are associated with chronic inflammatory diseases such as T2DM amongst others [241]. Clinically, increased concentration of OPN was previously observed in individuals with T2DM [20]. Whilst the generation of pro-inflammatory cytokines are associated with T2DM, anti-inflammatory cytokine, adiponectin (APN) is inversely associated with insulin resistance [17] and its immunosuppressive effect is mediated through the release of IL-10 [254]. Interestingly, adiponectin has been found to increase MMP-3 expression [378], suggesting a role in the interplay of inflammatory cytokines between OPN and MMPs.
The longer-term benefits of exercise in controlling weight, specifically decreasing visceral adipose tissue [379] and controlling blood glucose [64, 65] are well established. However, the response of APN, OPN and MMPs to exercise are largely unexplored in humans and existing research equivocal [298, 300, 380]. Moreover, despite the benefits of exercise, the role of exercise intensity on postprandial glucose is unclear for individuals at risk of developing T2DM. The purpose of this study therefore, was to determine the acute effects of exercise on the inflammatory markers MMP-2, MMP-3 and OPN along with APN, and concomitantly assess changes in the 48 h glucose response to two different types of exercise in overweight and sedentary individuals.
5.4 Methods

5.4.1 Study Design

The study followed a randomized cross-over design. All participants attended the Exercise Physiology labs at Murdoch University for a total of seven visits (introductory session; two experimental phases comprising three visits each). The introductory session (visit 1) involved consent process, a familiarization session and assessment of fitness (peak oxygen consumption, $V\dot{O}_{2peak}$; maximal aerobic power assessment). The two experimental phases comprised three study days each, with each phase completed in randomized order. The randomization sequence was generated using a computer generated random number list comprising 1’s and 2’s. The allocation of participants was conducted by a researcher not involved in the delivery of the intervention (TJF), by placing pre-numbered cards into opaque, sealed envelopes and recording the study ID’s. Participants were informed of the possible risks associated with the study before giving written informed consent. Ethics approval was granted by Human Research Ethics Committee at Murdoch University (Project number: 2013/121).

5.4.2 Participants

Fourteen sedentary (≤ 2 bouts of low-to-moderate intensity exercise per week) and overweight (BMI > 25 kg/m$^2$) males were recruited from the local community via advertisements posted on public noticeboards. Participants were deemed eligible if they were aged between 18 and 44 years old, had not been previously diagnosed with T2DM (fasting plasma glucose < 7 mmol/L) and had not smoked within the previous six months. Participants were excluded if they were taking anti-inflammatory medication, glucose controlling drugs (i.e. metformin), had uncontrolled hypertension, and had either an acute or chronic inflammatory disease/infection. Participants were pre-screened for exercise using the Exercise Science and Sport Science Australia (ESSA) medical screening tool.
5.4.3 Introductory Session

On arrival, participants were fully informed of all study procedures and written informed consent obtained. Body height, body mass and waist circumference measures were taken, and participants then shown the finger-stick procedure for collecting capillary blood for determination of blood glucose using a glucometer (Accu-Chek Go, Roche, Mannheim, Germany) required for the calibration of the continuous glucose monitor. Body composition was measured using the Dual Energy X-ray Absorptiometry (DXA; Hologic Discovery QDR series, Hologic Inc., Bedford, MA, USA). Thereafter, participants completed an incremental cycling test (50W; with 25W increments every 2 minutes) until volitional exhaustion on an electronically braked Velotron cycle ergometer (RacerMate, Seattle, WA, USA) for measures of VO$_2$peak. During the incremental cycling test, expired ventilation was collected as 15 s mean values using the Parvomedics TrueOne metabolic cart (Parvomedics, Sandy, UT, USA) and heart rate (Polar T31, Kempele, Finland) was collected at 1 Hz. Peak VO$_2$ was determined from the highest 30 s average VO$_2$ during the final stages of the incremental test. Heart rate and Borg’s [354] rating of perceived exertion (RPE) was collected throughout the maximal graded exercise test. This session was completed at least three days prior to the first experimental phase.

5.4.4 Experimental Trials

On day one of the experimental phase, participants reported to the laboratory (~0700 h) in a fasted state for the insertion of the continuous glucose monitoring system (CGMS; iPro2, Medtronic, Northridge, CA, USA). The sensor was inserted into an area of subcutaneous fat in the abdomen as recommended by the manufacturer. Following insertion of the sensor, participants were provided with a glucose meter (Accuchek Go, USA) in order to self-obtain finger-prick blood samples required to calibrate the glucose monitor over the three-day trial. In addition, participants were provided with an Activity Monitor (ActiGraph LLC, FL, USA) to assess physical activity patterns over the three-day period. An hour following insertion of the CGMS, an oral glucose tolerance test (OGTT; Carbotest, ThermoFisher, Australia) was completed (Day 1; baseline). Participants were then provided with a standardized breakfast (Up and Go, Sanitarium, Australia; two muesli bars, Kellogg’s, Australia) before leaving the laboratory. Food intake for the remaining portion of the day was recorded in a food diary and repeated during the second phase.
On day two, participants arrived at the laboratory at 0600 h fasted (overnight fast; water permitted). Venous blood sample was collected into EDTA and SST tubes (Becton Dickinson, NJ, USA) from the antecubital vein. Participants then, completed a warm up at 75W before completing the assigned exercise condition. Immediately upon the cessation of exercise, a second venous blood sample was collected, followed by an hour of recovery where participants were advised to relax and remain seated throughout the recovery period. A third venous blood sample was collected after the recovery period prior to the completion of an OGTT using the same methodology as the initial OGTT (day one) and at the same time of day (~0800 h). Thereafter, participants were provided with the standardized breakfast before leaving the laboratory.

On day three, participants reported to the laboratory in a fasted state prior to having the final venous blood sample collected and the final OGTT, which was performed at the same time as the previous days.

5.4.5 Exercise Conditions

Each of the two experimental phases followed an identical three-day design, with the exception being the type of exercise performed on day two. Day two comprised either a continuous moderate intensity exercise bout (CME; 30 minutes at 60% of $\dot{V}O_2^{peak}$), or a high intensity intermittent exercise (HIIE) bouts. The HIIE bout comprised six efforts at high- (1 minute at 100% $\dot{V}O_2^{peak}$) and low- (4 minutes at 50% $\dot{V}O_2^{peak}$) intensity which lasted 30 minute in duration. Participants were advised to maintain a cadence between 80-110 revolutions per minute (rpm) during the intense bouts and to maintain a comfortable cadence during the recovery period. This exercise protocol was based on a previously published protocol from our group [356]. Both exercise conditions proceeded with a standardized warm-up at 75W for five minutes. Heart rate and RPE were recorded throughout each exercise trial.

5.4.6 Blood Sampling

Serum samples (SST; 5mL) were allowed to clot for at least 30 minutes at room temperature, before being centrifuged at 1,300g for 15 minutes. Plasma samples (EDTA; 6ml) were spun immediately after collection at 1,300g for 15 minutes. The supernatant was then aliquoted in triplicate and stored at -80°C for later analysis. Samples were batch analysed using a multiplex cytokine assay for serum adiponectin (APN; Bio-Plex Pro Human Diabetes Assay); matrix metalloproteinase-2 (MMP2), MMP-3 and osteopontin (OPN; Bio-Plex Pro Human TH17 Assay) using the MAGPIX multiplex reader (Bio-Rad, Richmond, CA, USA).
5.4.7 Physical Activity Monitoring

A physical activity monitor (ActiGraph GT3X Activity Monitor; ActiGraph LLC, FL, USA) was provided to participants in order to record daily physical activity. Physical activity data were then assessed using the ActiLife software (version 5.6.1; ActiGraph LLC, FL, USA). Energy expenditure (kCal) over the three-day period was estimated by the algorithm incorporated within the software, specifically set for adults.

5.4.8 Data Analyses

Data are presented as mean ± SD, unless otherwise stated. Sample size was calculated with an effect size of $F = 0.3112$ with 80% power at a significance level of 5% based on IL-6 from Chapter 4. If sphericity was violated during the analysis, the Huynh-Feldt correction was used. Raw data from the CGMS and Actigraph were inspected for missing values and outliers prior to time synchronization and analysis. The raw values from the CGMS was evaluated as described below. In addition, the integrated area under the glucose curve (AUC-glucose) during the OGTT was calculated using the Trapezoidal rule (Graphpad PRISM) for each day and each condition, wherein the pre-OGTT value (time 0) was used as the baseline. Differences in blood analytes (MMP-2, MMP-3, OPN, APN), AUC-glucose, heart rate, RPE, mean-daily physical activity levels were analysed using a linear mixed model (LMM) with repeated measures (time), with one between factor (trial: CME vs HIIE) and one within factor (time: pre-exercise, post-exercise, 1h post-exercise and 25h post-exercise) modelled as fixed effects (intercept modelled as random effects). The primary outcome of interest was the time by condition interaction, while significant main effects of time were explored.

To avoid the problem of multiple point-wise comparisons over time, a bootstrapping analysis was used to assess the glucose values obtained from the CGMS. To this end, the blood glucose concentration at each time point on the comparator day (i.e., day 1 of each phase), was subtracted from the blood glucose concentration at each time point on the study day (i.e., Day 2 or Day 3 of each phase). This provided a difference or change score for each participant across the two days being compared, and enabled the calculation of a group-mean and 95% confidence interval (CI) bands over the course of the day. The change in glucose concentration was plotted against time (as mean ± 95% CI), wherein a significant response was defined as occurring when the lower 95% confidence limit for the curve was greater than zero, and a significant reduction in blood glucose was defined as occurring when the upper 95% confidence limit was less than zero [357]. All statistical analyses were completed using commercially available software (SPSS 24 Windows; SPSS, Chicago, IL, USA). Significance was accepted at $p \leq 0.05$.
5.5 Results

The participants in the study were: 27.4 ± 6.3 years old; classified as overweight or obese (BMI: 29.0 ± 3.2 kg/m²) with excessive adiposity (total body fat %: 27.2 ± 4.2%; visceral body fat: 613.2 ± 122.7 cm³); normoglycemic (fasting glucose: 5.2 ± 0.5 mmol/L); and had a VO₂ peak of 35.7 ± 5.1 ml/kg/min. All participants completed the two experimental phases. One participant completed only five sprints during the HIIE bout, whilst one other participant completed all six sprints in the HIIE bout, but did not complete the last 4 minutes (low-intensity portion of the protocol) of the bout. CME elicited a mean-sessional heart rate and RPE (mean ± SD) of 137 ± 25 beats per minute (bpm) and 14 ± 2, respectively; whilst HIIE elicited a significantly higher mean-sessional heart rate and RPE (151 ± 33 bpm and 16 ± 2, respectively).

All subjects wore the activity monitor over the three-day period. Energy expenditure (kCal), total time of sedentary (Sed Time) and the vector magnitude (VM) was no different between days (p > 0.178 for all variables) or condition (p>0.196 for all variables). Data displayed in Table 5.1.

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Data as mean ± SD. There were no significant differences. Energy expenditure expressed as kilocalories (kCal); Time spent in sedentary expressed as Sed. Time in minutes; total vector magnitude expressed as VM. Counts per minute, expressed as cpm; time expressed as minutes (min).

Table 5.1. Physical activity monitor measurement
5.5.1 Inflammatory Cytokines

Data from ten participants were available for analysis of the inflammatory cytokine panel (Figure 5.1). There were no time by condition interactions observed for the markers of interest (MMP-2: p = 0.876; MMP-3: p = 0.886; OPN: p = 0.663; APN: p = 0.778). A main effect of time for MMP-3 (p = 0.003) and OPN (p = 0.006) were observed (Figure 5.1). Exercise resulted in an acute increase in MMP-3, which was ablated by 1h post-exercise. In contrast, OPN decreased significantly in response to exercise, returning to pre-exercise levels by 25h post-exercise (Figure 5.1).

![Figure 5.1](image)

**Figure 5.1.** Mean (± SD) concentration of matrix metalloproteinase-2 (MMP-2), matrix metalloproteinase-3 (MMP-3), osteopontin and adiponectin. MMP-3: a. Significantly different than Pre (p=0.001) and 25 h (p=0.001). Osteopontin; a. Significantly different than Pre (p = 0.006) and 25 h (p = 0.001). No differences were found with adiponectin and MMP-2. CME (open bars) vs HIIE (hatched bars).
5.5.2 Glucose: Continuous Glucose Monitoring

Following the evaluation of data from the CGMS, data from 3 participants were excluded due to significant (>4 hours) lapses in the measurements. There was a main effect of time (p = 0.037) on mean-daily glucose values, with the average glucose concentration on Day 3 being higher than Day 1 (Glucose: 5.6 ± 0.2 vs Glucose: 5.2 ± 0.1 mmol/L; p = 0.050), but there was no significant interaction effect (p = 0.755; Figure 5.2). There were only minor periods throughout the day, where significant differences between study days were observed as indicated by the upper- or lower-95% CI bands crossing zero (Figure 5.3).

The glucose profile from the CGMS during the OGTT revealed a main effect of time (p <0.001), but no condition by time interaction effects (p = 0.840; Figure 5.2). The AUC-glucose revealed a significant effect of time (day; p=0.027) but no interaction effect (p = 0.078). The AUC for glucose on Day 1 was significantly lower than day 2 (mean difference: 0.28 mmol/L/min; value: p = 0.043) and day 3 (mean difference: 0.46 mmol/L/min; p = 0.008).
Figure 5.2. (Top) Mean (±SD) glucose concentration over each day calculated from CGMS. a, significant difference between Day 1 and Day 3, p = 0.05. (Bottom) Glucose concentration during OGTT on each day. A main effect of time over the OGTT was present, p < 0.001 and a main effect between days was present, p = 0.025. Mean glucose was lower on Day 1 vs Day 3, p = 0.043. CME (open bars) vs HIIE (hatched bars).
Figure 5.3. Mean differences (with 95% CI) in 24h glucose concentrations between A: HIIE on Day-2 minus Day-1; B: CME on Day-2 minus Day-1; C: HIIE on Day-3 minus Day-1; D: CME on Day-3 minus Day-1. Subplot next to each graph represents mean glucose difference concentration and standard deviation over each respective 24 h period. Arrow indicates when exercise was completed.
5.6  Discussion

Emerging roles for MMP-2, MMP-3 and OPN in the development of adipose tissue and more broadly T2DM are now being elucidated [21, 23, 24, 48-50]. The response of OPN and MMPs to exercise are largely unexplored in humans and existing research equivocal [380]. We sought to determine the blood glucose response and changes in circulating MMP-2, MMP-3 and OPN, to two different exercise types in overweight and sedentary individuals. The main findings were i) exercise did not improve diurnal glucose profiles as demonstrated by similar glucose profiles across the day; and indeed appeared to inflate the glucose response; (ii) the glucose profile during the OGTT supported the diurnal pattern, with the AUC performed 1 h after exercise (Day 2) and 25 h after exercise (Day 3) being significantly higher than Day 1; iii) MMP-3 was acutely increased in response to exercise while MMP-2 did not demonstrate significant changes in response to exercise; iv) OPN was acutely decreased following exercise, and returned to baseline levels by 25h.

Osteopontin appears to play an important initiating role in adipose-tissue expansion, inducing the expression of MMP-2 via pro-MMP-2, and inflammation [20, 52]; while MMP-3 appears to have an inhibitory effect on adipocyte differentiation [50]. The decrease in OPN concentration immediately post-exercise however, did not correspond with a significant decrease in MMP-2 concentration. Although previous findings of reduced OPN in mice was beneficial in reducing adipose tissue inflammation and improving glucose tolerance [241, 381], the reduction in OPN in the current study was only transient and appeared not to have an effect on glucose tolerance. Indeed, while the respective response of these markers to exercise are consistent with potentially longer-term health benefits; in the current study, these changes were not associated with changes in diurnal glucose profiles as evidenced by a lack of significance over time and the higher glucose responses 1 h and 25 h post-exercise (Figure 5.2 and 5.3).

To our knowledge, this is the first study that has investigated the effect of exercise intensity on both MMP-2 and MMP-3 in overweight/obese sedentary individuals. The increase in MMP-3 immediately post-exercise was similar to Urso et al. [382] who also found a transient increase in MMP-3 immediately following acute exercise. While decreased MMP-3 is observed over the longer term with exercise training in diabetic mice [303], evidence in humans remain limited. Previous cell culture work [378] found that APN induced the expression of MMP-3 which may explain the increase in MMP-3 observed immediately post-exercise. However, the present findings did not see changes in APN regardless of condition or day. The acute effect of exercise on APN remain equivocal with increases observed following
acute moderate-to-high intensity (55% to 70% $\dot{V}O_{2\text{max}}$) aerobic exercise [300, 383], whilst others have not seen changes following moderate-intensity (60-65% $\dot{V}O_{2\text{max}}$) aerobic exercise [298, 384].

Physical inactivity has been shown to impair glycemic control [207]. As a consequence, the assessment of postprandial glucose is crucial where if elevated, can lead to the development of type 2 diabetes [385]. Elevated postprandial glucose is also an independent risk factor for cardiovascular events in individuals with or without type 2 diabetes [386]. Fortunately, poor glycemic control can be ameliorated following increased physical activity [207]. However, despite the improvements seen in blood glucose following acute exercise [207, 387], neither a single bout of CME or HIIE in the present study was sufficient in improving glycemic control as seen from a lack of improvement in 24 h and 48 h blood glucose upon cessation of exercise (Figure 5.3). Our findings were in contrast to the findings of Oberlin et al whom had found improved glucose response in the 24 h following acute exercise, but not in the subsequent 24 h in type 2 diabetic individuals [334]. Interestingly, despite a ~10% reduction in glucose response during the first 24 h, significantly lowered postprandial glucose AUC response was only found following one of three meals [334]. In addition to improvements seen 2.5 h post-breakfast glucose on the first day of exercise, van Dijk and colleagues found that a single bout of exercise was consistently reduce post-breakfast glucose on the second day following exercise [388]. Some differences of the current study may be attributed to our population of interest which did not present with T2DM, resulting in less room for improvement of glycemic control. Secondly, the present study utilized OGTTs for the glucose challenge, as opposed to mixed meals used in both studies where its macronutrient distribution may have affected glycemic responses. The timing of exercise and the subsequent meal may have also affected glucose responses such that in the present study the OGTT was consumed in a fasted state 1 h following exercise rather than 2 h following exercise [334]. In the study by van Dijk et al. exercise was performed following breakfast, which may have further altered the glucose response following exercise [388]. Physical activity throughout both trials were monitored using a tri-axial activity monitor over each three-day period. Physical activity monitoring found that energy expenditure, sedentary time and total vector counts were no different between exercise conditions and days indicating that neither conditions promoted an increase
in activity throughout each day regardless of exercise intensity.

This study had a number of strengths including i) duration- and workload-matched CME and HIIE conditions; ii) recruiting a clinically relevant population, namely individuals who were inactive and overweight- which has, to our knowledge not previously been conducted in a study of this kind; iii) adopting a randomized cross-over design; iv) each trial included a specific baseline compromising of 24h continuous glucose monitoring which were used to compare 24h differences following exercise for two days post exercise. However, there were also some limitations associated with this study. Although the use of the CGMS provides valuable information on the rapid short-term changes in glycaemic fluctuations, it does not directly measure glucose concentrations. Rather, it measures a signal that is proportional to the glucose level in the interstitial fluid. Another limiting factor previously experienced included the time lag between the variation of blood glucose and those from the subcutaneous interstitial fluid [389-391]. Errors were minimized by only using two continuous glucose monitors throughout the study, with each subject using the same unit for both phases. Despite the measurement of MMP-2 and MMP-3 in this study, we did not measure activity of tissue inhibitors of MMPs which would have accounted for the regulatory activity of these MMPs which may have provided further insight into the acute effect of MMPs following exercise. Additionally, although exploring the changes of inflammatory markers between exercise-intensity provides an insight on the acute effect of exercise, implementing this study in a type 2 diabetic population would be a strength for future studies that would have allowed assessment across clinical and pre-clinical stages. Although the strict timing on completion of the OGTT allowed for consistency over each trial, providing all participants with standardized meal over the three days at strict meal times would have allowed for greater control of dietary intake. Finally, conducting multiple regression analysis to assess the effect of respective markers on glucose profile would provide more substantial and concrete answers, however a greater number of participants would be required to run such an analysis. Due to the lack of significant change in the glucose profile following exercise, we were not able to determine the impact of the changes in these inflammatory markers on the glucose response.
The present study demonstrated that a single bout of moderate- and high-intensity intermittent exercise was effective in inducing changes to MMP-3 and OPN immediately following exercise which is consistent previous work, although this did not persist and returned to baseline levels by the next morning. An improvement in glucose tolerance was not evident following completion of OGTTs over the three days which is in contrast to previous findings, which was also reflected in continuous glucose concentrations in the 48 h period following exercise. Further research is warranted to address limiting factors such as glycemic status, exercise and meal timing (preprandial vs postprandial) across an at-risk population as well as clinically diagnosed type 2 diabetes individuals.

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**Author Contributions**

A.R, T.J.F, J.J.P conceptualized and designed the study; A.R. and N.G.L performed experiments; A.R and N.G.L analysed data; A.R, G.F.H and T.J.F interpreted results of experiments; AR and T.J.F drafted manuscript, A.C, A.R, G.F.H, J.J.P, N.G.L and T.J.F edited and revised manuscript. All authors approved the final version of manuscript.
Chapter 6  Short-term training effect on glycaemic control and the inflammatory response in type 2 diabetes.

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6.1 Abstract

This study examined the effect of short-term training on glycaemic control and the corresponding inflammatory response associated with type 2 diabetes mellitus (T2DM). Thirteen (5m/8f) obese (32.0±4.7kg/m²) individuals previously diagnosed with T2DM completed 12 exercise training (45 minutes of treadmill exercise; 65% VO₂peak) sessions in successive days. An oral glucose tolerance test (OGTT; 75g) was completed before and 48 h after the training period, along with fasting plasma measurements of fructosamine, glucose, insulin, and C-Reactive Protein. To distinguish the acute and training responses, inflammatory cytokine (unstimulated and LPS-stimulated) responses immediately following the first and last exercise session were assessed in addition to the measurement of regulatory T cells which are of anti-inflammatory nature. The acute response of exercise resulted in increased pro- (IL-1β, TNFα and IP-10) and anti-inflammatory (IL-10) cytokine response following the last training session (p≤0.045); however, no concurrent differences were observed in regulatory T cells at any time point (p>0.650). Although an improvement in glucose and insulin area under the curve was not evident (p>0.135), exercise training was accompanied with a 5% reduction in glycaemic marker, fructosamine (p=0.023) which was concomitant with reduced (~27%) global inflammation measured from CRP (p=0.037). Although exercise training was effective in improving glycaemic control and reducing global inflammation as measured from CRP, the acute increase in cytokine response indicates that longer term training may be of benefit to demonstrate a reduced inflammatory response as a result of the training adaptation which would be expected with a greater proportion of regulatory T cells.

Trial Registration: ACTRN12615000613505
6.2 Introduction

Maintaining an appropriate balance between anti-inflammatory and pro-inflammatory cytokines in the systemic circulation appears critical to avoiding inflammatory-related disease, including type 2 diabetes (T2DM) [263]. To this end, adipose tissue in lean individuals is largely comprised of alternatively-activated macrophages (M2) which promote a Th2-type response producing anti-inflammatory cytokines such as Interleukin (IL)-10 and IL-1 receptor antagonist (IL-1ra) [198]; balancing the pro-inflammatory cytokines produced by the classically-activated (M1) macrophages [198, 392]. The anti-inflammatory environment of the adipose tissue is further supported by regulatory T cells (Tregs) through the production of IL-10 and Transforming Growth Factor-beta (TGF-β) [392, 393]. The increased accumulation of adipose tissue associated with obesity facilitates an increased shift in the polarization of these macrophages towards the M1-like phenotype [198]. This increased M1 infiltration and subsequent increase in pro-inflammatory cytokines is associated with the characteristic insulin resistance in obesity-linked T2DM [12, 14, 197]. Concomitantly, there are a reduced number of adipose tissue resident Tregs associated with obesity and T2DM, and the functional suppressive capacity of these cells are reduced [263]. Accordingly, increased circulating concentrations of acute phase proteins such as C-reactive protein (CRP) and alpha-1 acid glycoprotein (AAGP), as well as classic pro-inflammatory cytokines such as IL-1β, TNFα, and IL-6 are characteristic in individuals with obesity and T2DM [394-396]. Furthermore, evidence suggest that Monocyte Chemoattractant Protein (MCP-1) and IL-8, IL-15 and Interferon Gamma-Induced Protein 10 (IP-10) may also contribute to insulin resistance. MCP-1 is a pro-inflammatory chemokine that has been shown to promote macrophage infiltration and insulin resistance in mice [397]. MCP-1 may also play a significant role in insulin resistance by affecting insulin sensitivity in adipocytes which reduced insulin-stimulated glucose uptake following incubation of a low-dose of MCP-1 in vitro [398]. IL-8 and IP-10 are also chemokines which have been previously found to positively associate with insulin resistance and T2DM [399, 400]. Importantly, both these markers are also reported to associate with hsCRP and fasting glucose which may prove to be a significant risk marker of T2DM [399, 400]. On the other hand, IL-15 is a growth factor produced in skeletal muscle which has been found to stimulate adiponectin, an adipose-derived anti-inflammatory cytokine known to promote insulin sensitivity [401]. Indeed, previous work has supported this theory as seen in obese mice following IL-15 treatment which resulted in improved insulin sensitivity [402]. Therefore, systemic low grade chronic inflammation is not only believed to be characteristic of T2DM, but is attributed to play an important role in the development of obesity-related insulin resistance and T2DM [195, 403].
Exercise is recommended as a key component in the management of individuals with T2DM [404]. Exercise increases the translocation of glucose transporters (GLUT4) to the plasma membrane promoting increased glucose uptake acutely [32, 272, 273, 387, 405-407]. In the longer-term, exercise training in individuals with T2DM results in increased GLUT4 content and improved glucose tolerance [184], and is associated with significantly reduced glycated haemoglobin (HbA1c) [27, 408], a marker of glycaemic control. This improvement in HbA1c is comparable to the use of non-insulin lowering medication [28, 408]. Of relevance, training studies of shorter duration (i.e., <12 weeks) observed a 0.8% decrease in HbA1c which was more pronounced than studies of longer duration (i.e., >12 weeks; 0.7%) [409]. These improvements may be partially attributed to increased exercise volume and the specific progressive nature of the exercise training adopted [409]. Subsequent short-term training studies from as few as six sessions [33] to seven days of consecutive training [40, 182] have reported improvements in glycaemic control, which is likely explained by improved insulin action [41, 188].

Acute exercise elicits an exaggerated inflammatory response [410, 411] while exercise training attenuates this response, although large heterogeneity in responses are seen between populations and training variables [410]. Exercise training in obese non-T2DM [205, 206] and T2DM appears to have beneficial effects on the inflammatory status such as decreased concentration of global marker of inflammation, hsCRP (high-sensitivity CRP), as well as decreased inflammatory cytokines including IL-1β, TNFα and increased IL-10 [29, 44]. Whether changes in inflammatory markers are observed concurrent with improvements in glycaemic control in individuals with T2DM in response to short-term training, is not known. Therefore, this study sought to determine the impact of 12 days of continuous exercise on glycaemic control, changes in inflammatory cytokines and the population of regulatory T cells. Glycaemic control was assessed using fructosamine, which is a short-term (~2 weeks) marker of glycaemic control [412], and glucose response during an oral glucose tolerance test. We hypothesised that exercise training would be associated with i) decrease in fructosamine and improved glucose tolerance; ii) decrease in pro-inflammatory markers and an increase in anti-inflammatory markers. A secondary aim was to assess the acute inflammatory response to exercise, by assessing inflammatory markers pre- and post-exercise on the first and last (12th) exercise session. To aid the interpretation of changes in the circulating inflammatory markers, we also assessed the direct functional ability of immune cells to produce an inflammatory response in vitro. We hypothesised that the inflammatory response would be attenuated in the 12th exercise session relative to the first session, but the inflammatory response to in vitro stimulation (using a toll-like receptor 4 ligand) would be unaffected.
6.3 Methods

6.3.1 Study Design

This study adopted a single-arm, repeated measures design. All participants attended the Exercise Physiology laboratory at Murdoch University for a total of sixteen visits over a three-week period. The first visit involved the consent process, a familiarisation session and assessment of fitness (peak oxygen consumption, \( \dot{V}O_{2\text{peak}} \) assessment). At least two days following familiarisation, participants visited the laboratory in the morning for the completion of a baseline oral glucose tolerance test (OGTT) before commencing the first of twelve training session the next day. Following the two-week training period, an OGTT was repeated 16-24h following the last training session. In addition to the blood sampling during the OGTT, venous blood samples were collected via venepuncture prior to and immediately following the first and last exercise sessions during the training period. Participants were informed of the possible risks associated with the study before providing written informed consent. Ethics approval was granted by Human Research Ethics Committee at Murdoch University. This trial was prospectively registered with Australian New Zealand Clinical Trials Registry: ACTRN12617000286347.

6.3.2 Participants

Thirteen (5m / 8f; aged 51 ± 7 y) sedentary, overweight/obese (BMI > 27 kg/m\(^2\)) volunteers previously diagnosed with T2DM (HbA1c range; 6.2% to 10.2%) were recruited from the local community (Table 6.1). Participants were deemed eligible if they were between 18 and 65 years old, and completed less than or equal to two bouts of low-to-moderate intensity exercise per week). Exclusion criteria included the use of exogenous insulin, contraindications to exercise training and smoking. Participants were pre-screened for exercise using the Exercise Science and Sport Science Australia (ESSA) medical screening tool. 12 participants were on anti-diabetic medication, 4 were on anti-hypertensive medication and 2 were on anti-depressive medication.
<table>
<thead>
<tr>
<th></th>
<th>Pre-training</th>
<th>Post-training</th>
<th>Effect of training (p-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>51 (7)</td>
<td>51</td>
<td></td>
</tr>
<tr>
<td>HbA1c (mmol/mol)</td>
<td>53.8 (21.5)</td>
<td>53.8</td>
<td>ES: 0.007; p = 0.809</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.66 (0.07)</td>
<td>1.66</td>
<td>ES: 0.000; p = 0.931</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>90.6 (14.8)</td>
<td>90.7 (14.6)</td>
<td>ES: 0.244; p = 0.134</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>32.7 (4.9)</td>
<td>32.7 (4.6)</td>
<td>ES: 0.129; p = 0.051</td>
</tr>
<tr>
<td>WC (cm)</td>
<td>103.9 (11.9)</td>
<td>101.1 (10.3)</td>
<td>ES: -0.034; p = 0.450</td>
</tr>
<tr>
<td>Fat (%)</td>
<td>37.1 (6.4)</td>
<td>36.2 (7.1)</td>
<td>ES: -0.066; p = 0.491</td>
</tr>
<tr>
<td>VAT (g)</td>
<td>901.6 (340.3)</td>
<td>925.8 (366.9)</td>
<td></td>
</tr>
<tr>
<td>VAT (cm³)</td>
<td>974.7 (367.9)</td>
<td>1000.9 (396.6)</td>
<td></td>
</tr>
<tr>
<td>V̇O₂peak (ml/kg/min)</td>
<td>23.1 (4.9)</td>
<td>24.1 (4.7)</td>
<td>ES: -0.202; p = 0.011</td>
</tr>
</tbody>
</table>

Table 6.1. Descriptive characteristics of the participants at baseline (data presented as mean (SD)) and changes in anthropometric measures in response to exercise training; where effect of training is represented by Cohen’s 𝑑 effect size and 𝑝-values. BMI, body mass index; WC, waist circumference; VAT, visceral adipose tissues; V̇O₂peak, peak VO₂ achieved.

### 6.3.3 Introductory session

On arrival, participants were fully informed of all study procedures and written informed consent obtained. Body height, body mass, waist circumference was measured, whilst body composition was assessed using Dual Energy X-ray Absorptiometry (DXA; Hologic Discovery QDR series, Hologic Inc., Bedford, MA, USA). Thereafter, participants completed a cardiorespiratory fitness test (start at 2.5km/h, 1km/h ramp every 2 minutes until 5.5km/h followed by 2% increase in gradient thereafter) until volitional exhaustion on a treadmill (Trackmaster, Full Vision, Kansas, USA) for measure of V̇O₂peak. During the cardiorespiratory fitness test, expired ventilation was collected in 15-second averages using a metabolic cart (Parvomedics TrueOne, Sandy, UT, USA) and heart rate (Polar T31, Kempele, Finland) was collected at 1 Hz. V̇O₂peak was determined as the highest 30s average V̇O₂ from the final stage of the ramp test. Heart rate and Borg’s (6) rating of perceived exertion (RPE) was collected throughout the maximal graded exercise test. This session was completed at least three days prior to the first training session. These tests were repeated upon completion of the training period.
6.3.4 Oral Glucose Tolerance Test (OGTT)

OGTTs (75 g glucose) were performed after an overnight (10–12 h) fast at the same time of day before and following the training period. Participants refrained from exercise and medication use on the morning of testing. Post-intervention OGTTs were performed within 36-48 h of the final bout of exercise. Prior to the ingestion of the glucose-containing drink, a catheter was placed in an antecubital vein for venous blood samples collected at 30 min intervals for measurement of glucose and insulin. Food intake prior to each OGTT was at the participants’ discretion and were advised to refrain from dense sugar containing foods in the evening prior to the OGTT. Participants recorded their food intake in a 24h food recall and were advised to replicate their meal to their best ability prior to the OGTT post training.

6.3.5 Experimental Phases (Exercise intervention)

On 12 consecutive days, all volunteers performed 45 min of supervised exercise for 12 consecutive days at ~65% VO$_2$peak. A heart rate monitor was worn during each session to monitor heart rate corresponding to the desired workload from the cardiorespiratory fitness test. All sessions were completed from participants with 100% compliance.

6.3.6 Biochemical analysis

6.3.6.1 Glycated haemoglobin (HbA1c) and high sensitivity CRP (hsCRP)

Fasting venous blood samples were collected into EDTA and lithium heparin tubes for the analysis of HbA1c and hsCRP a local commercial laboratory (Western Diagnostics Pathology, Myaree, Western Australia). HbA1c was measured pre-training whilst hsCRP was collected pre- and post-training (within 36-48 h of the final bout of exercise).

6.3.6.2 Glucose, Insulin, inflammatory markers

Blood samples from the OGTT were collected into EDTA tubes containing aprotinin (50KIU/ml blood; Becton Dickinson, Plymouth, United Kingdom) and serum clot activator tubes (Becton Dickinson, Plymouth, United Kingdom). The EDTA tube was immediately centrifuged at 1300g for 10 minutes whilst serum tubes were allowed to clot for 60 minutes before centrifuging at the same speed. Plasma and serum samples were aliquoted into triplicates and stored at -80°C for later analysis. Alpha-1 acid glycoprotein, blood glucose and fructosamine were assessed via chemical analyser (COBAS Integra 400 plus, Roche
Diagnostics Ltd, Switzerland) and insulin by enzyme linked immunoassay (Mercodia; Uppsala, Sweden). The area under the curve (AUC) for glucose and insulin during the OGTT were calculated using the trapezoidal method. Insulin resistance was calculated using the homeostasis model assessment (HOMA) of insulin resistance (HOMA2-IR), β-cell function (HOMA2%beta) and insulin sensitivity (HOMA2%S) from fasting glucose and insulin concentrations (HOMA2 calculator (v2.2); available online at http://www.dtu.ox.ac.uk/homacalculator/), the insulin sensitivity index (ISI) composite was calculated using glucose and insulin values at the 0 and 120 min from an OGTT [413]. Additionally, the hepatic insulin resistance index was calculated using the 0 and 30min glucose and insulin values; although fasting indices are thought to reflect hepatic insulin resistance, this measure is believed to be more specific to hepatic insulin resistance [414]. To assess peripheral insulin sensitivity, the Cederholm index was calculated as previously described [415].

6.3.6.3 Toll-like receptor stimulation and blood culture

Venous blood samples were collected via venepuncture prior to and immediately following the first and last (12th) exercise session during the training period. The in vitro inflammatory response to toll-like receptor (TLR) stimulation was assessed by adding 180 μL of diluted blood to each well in a 96-well plate containing the TLR4 ligand, prepared as previously described at the University of British Columbia in Vancouver, Canada [416]. The plates contained the TLR4 ligand, lipopolysaccharide (LPS; TLR-4, InvivoGen) at 10 ng/mL. The control condition consisted of a 96-well plate which did not contain LPS. Following addition of blood, plates were incubated at 37°C in 5% CO₂ for 24 hours after which the plates were centrifuged at 600g and 50 μL of supernatant was removed and stored at -80°C for later analysis.

6.3.6.4 Regulatory T cells (Tregs)

Venous blood collected via venepuncture prior to and immediately following the first and last exercise session were stained for Tregs using fluorescent-conjugated monoclonal antibodies to identify cell surface (CD3-BV510, CD4-APCH7, CD8-PerCpCy5.5, CD25-PeCy7, CD127-BV421, CD120B-PE and live/dead stain-FITC) and intracellular (Foxp3-AF647) markers via eight-colour flow cytometer (BD FACS CantoII, Becton Dickinson, UK). Antibodies were mixed in 150μL of whole blood containing EDTA into a TruCount tube (Becton Dickinson, UK) and set to incubate for 20 minutes on ice. Red blood cells were then lysed with lysing solution (BD Pharmlyse, Becton Dickinson, UK) following further incubation for 15 minutes
before being spun at 600g at 4°C for 5 minutes and washed twice using phosphate-buffered saline (PBS). The supernatant was then aspirated and cells were resuspended in PBS followed by incubation with a live/dead stain (Zombie Fixable Viability kit, Biolegend, CA, USA) for 15 minutes. Cells were then washed twice and resuspended in PBS before fixation and permeabilisation (Foxp3 fix/perm buffer set, Biolegend, CA, USA) as per manufacturer’s recommendations for the intracellular staining of foxp3. 150uL of stabilizing fixative (Becton Dickinson) was then added prior to flow cytometry analysis within 24h. Forward-scatter versus side-scatter plot was used to gate lymphocytes based on size and density. Live cells were included from the live/dead stain followed by the inclusion of single cells by gating SSC-W by SSC-H. Viable single cells were then gated for CD3+CD4+CD8- T cells of which Tregs were identified by further gating of CD127-CD25+ cells (Figure 6.1). The FoxP3, CD25 and CD120B gates were set using FMO controls in order to correctly identify a positive population. From this population, confirmation of Tregs were gated by foxp3. Absolute cell counts were calculated from TruCount tubes by dividing the number of positive cell events (X) by the number of bead events (Y), and then multiplying by the BD Trucount bead concentration (N/V, where N = number of beads per test* and V = test volume); A = X/Y × N/V.

6.3.6.5 Cytokine measurement

Supernatants were thawed at room temperature and assayed by multiplex assay. The high-biotin protocol was used to measure the levels of the following cytokines: IL-10, IL-15, IL-1β, IL-6, IL-8, IP-10, MCP-1, TNF-α (eBioscience custom multiplex, Jomar Life Research, Australia) and TGF-β were measured from a ProcartaPlex simplex bead set (eBioscience catalog, EPX01A-10249-901, Jomar Life Research, Australia) which was combined with the Procarta Human Basic Kit (eBioscience, catalog EPX010-10420-901, Jomar Life Research, Australia) All assays were measured using the Bioplex system (BioRad, Hercules, CA, USA) running the Bioplex analysis software.

6.3.7 Data Analyses

Data is presented as mean ± SD, unless otherwise stated. Sample size was calculated with an effect size of F = 0.4326 with 80% power at a significance level of 5% based on Foxp3. If sphericity was violated during the analysis, the Huynh-Feldt correction was used. Glucose and insulin concentrations from the oral glucose tolerance test were used to calculate the area under the curve (AUC) using Graphpad software (Graphpad, PRISM). The inflammatory cytokine panel, cell populations and acute phase reactants were analysed using a two-way analysis of variance (ANOVA) with repeated measures; with one between factor (Training: 1st session vs
12th session) and one within factor (acute exercise; pre-exercise, post-exercise). The hypothesis of interest was the interaction effect (training x acute exercise), however, main effects were explored with post Hoc analysis using least squared differences. Paired student’s t-test were calculated for pre- and post-training time points for anthropometric variables, $\dot{V}O_{2peak}$, HOMA2-related outcomes, glucose-AUC, insulin-AUC, hsCRP and fructosamine. Pearson’s correlation was calculated to determine relationships between variables of interest (hsCRP, body fat percentage and $\dot{V}O_{2peak}$). All statistical analyses were completed using commercially available software (SPSS 21 Windows; SPSS, Chicago, IL, USA). Significance was accepted at $p \leq 0.05$.

**Figure 6.1.** Identification of Tregs via flow cytometry.
6.4 Results

All participants completed twelve training sessions with 100% compliance. Due to difficulties with blood samples before and after acute exercise on the 1st and 12th session, data was only available from 9 of 13 participants (4m/5f) at these time points. Participants were on average, well controlled (HbA1c: 53.8 mmol/mol; 7.1%), although ten participants had HbA1c over 6.5% and four had HbA1c over 8%.

6.4.1 Fitness and Anthropometric data (Table 6.1)

All participants completed the \( \dot{V}O_2^{\text{peak}} \) test with a respiratory exchange ratio of greater than 1.10 and \( \dot{V}O_2 \) reaching a plateau at the end of the cardiorespiratory fitness test. Following 12 consecutive days of treadmill exercise, participants increased \( \dot{V}O_2^{\text{peak}} \) (p = .011; \( d = 0.202 \)) and reduced body fat percentage (p = .051; \( d = 0.129 \)). There were no significant changes in body weight or BMI (both p ≥ .809), and there were no significant changes in waist circumference or visceral adipose tissue (volume and weight) (all p ≥ .134; \( d ≥ 0.244 \); Table 1).

6.4.2 Glycaemic variables (Table 6.2)

Fructosamine was significantly (p = .023; \( d = 0.202 \)) decreased following the 12 days of exercise training (Table 2). However, exercise training did not result in significant differences in fasting blood glucose, 2 h glucose AUC or 2 h insulin AUC (All p ≥ .135; All \( d ≤ 0.18 \)). Indices of insulin sensitivity, indicative of total, hepatic and muscle insulin sensitivity, were also not significantly altered after the short-term training period (All p ≥ .086; All \( d ≤ 0.5 \)).
### Glycaemic control

<table>
<thead>
<tr>
<th></th>
<th>Pre-training</th>
<th>Post-training</th>
<th>Effect of training (p-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fructosamine (µmol/L)</td>
<td>307 (76.6)</td>
<td>291.7 (67.4)</td>
<td>ES: 0.202; p = 0.023</td>
</tr>
<tr>
<td>FBG (mmol/L)</td>
<td>10.3 (3.9)</td>
<td>9.8 (4.0)</td>
<td>ES: 0.123; p = 0.301</td>
</tr>
<tr>
<td>2h Glucose AUC (mmol/L)</td>
<td>1931 (514)</td>
<td>1830 (590)</td>
<td>ES: 0.177; p = 0.135</td>
</tr>
<tr>
<td>2h Insulin AUC (µU/mL)</td>
<td>4820 (3909)</td>
<td>4464 (3522)</td>
<td>ES: 0.007; p = 0.225</td>
</tr>
</tbody>
</table>

### Insulin resistance

<table>
<thead>
<tr>
<th></th>
<th>Pre-training</th>
<th>Post-training</th>
<th>Effect of training (p-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HOMA2-IR</td>
<td>2.48 (1.44)</td>
<td>2.26 (1.24)</td>
<td>ES: 0.159; p = 0.134</td>
</tr>
<tr>
<td>HOMA2-B (%)</td>
<td>58.5 (51.8)</td>
<td>60.2 (49.2)</td>
<td>ES: -0.017; p = 0.554</td>
</tr>
<tr>
<td>HOMA2-S (%)</td>
<td>49.0 (18.0)</td>
<td>54.1 (21.6)</td>
<td>ES: -0.248; p = 0.113</td>
</tr>
<tr>
<td>ISI (composite)</td>
<td>2.24 (1.01)</td>
<td>2.38 (1.02)</td>
<td>ES: -0.069; p = 0.331</td>
</tr>
</tbody>
</table>

### Peripheral insulin resistance

<table>
<thead>
<tr>
<th></th>
<th>Pre-training</th>
<th>Post-training</th>
<th>Effect of training (p-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Matsuda muscle</td>
<td>-0.113 (0.103)</td>
<td>-0.079 (0.090)</td>
<td>ES: -0.174; p = 0.301</td>
</tr>
<tr>
<td>Cederholm</td>
<td>21.4 (10.7)</td>
<td>24.4 (12.6)</td>
<td>ES: 0.499; p = 0.086</td>
</tr>
</tbody>
</table>

**Table 6.2.** Measures of glycaemic control and insulin resistance pre- and post-training (presented as mean (SD)) with associated effect (Cohen’s d) of the exercise training intervention. FBG, Fasting blood glucose; AUC, Area under the curve; HOMA2-IR, Homeostatic model assessment index 2; HOMA2-B, beta cell function; HOMA2-S, insulin sensitivity; ISI, Insulin sensitivity index

#### 6.4.3 Acute phase proteins and hsCRP

Exercise training was associated with a significant decrease in hsCRP (27.2%; p = 0.037; Figure 6.2). Although the change in hsCRP did not correlate with changes in body fat percentage (r = -0.259; p= 0.202) or VO₂peak (r = 0.156; p = 0.446). A significant interaction effect in the acute phase protein alpha-1 acid glycoprotein (AGP; p = 0.001) was identified, wherein the acute response (pre- and post- exercise session) was altered following the 12 consecutive training bouts. Specifically, the pre-exercise AAGP concentration was ~5.2% lower at the 12th exercise session compared to the 1st exercise session; while the concentration of AAGP post-exercise were comparable between sessions (Figure 6.2). No significant differences were found for homocysteine over the training period (p = 0.233).
Figure 6.2. Mean (± SD) concentration of acute phase reactants; Alpha 1-acid glycoprotein, Homocysteine, Fructosamine, high sensitivity C-reactive Protein and Transforming Growth Factor-beta. *, post-exercise was greater than pre-exercise in the 12th session, p < 0.001; ^, Post-training was lower than pre-training values, p = 0.037.

6.4.4 Cell populations (Table 6.3)

Exercise was not associated with changes in either absolute cell count or relative percentage of CD3+CD4+ T cells or CD3+CD8+ T-cell populations, either in response to acute exercise (pre- versus post-exercise session) or training (1st session to 12th session; all main effects p ≥ 0.483, Table 3). This highlighted that exercise did not induce further immune activation following exercise as demonstrated by no changes in proportionality in either CD3+CD4+ or CD3+CD8+ T cell production. Additionally, the ratio of CD4+ to CD8+ was not altered by acute exercise (p = 0.842) or exercise training (p = 0.462). Unsurprisingly, the lack of changes in the T-cell population was also reflected in the percentage of CD4+CD127-CD25+ Tregs and CD4+CD127-Foxp3+ Tregs which were not affected by neither acute exercise nor training (p > .650; Table 6.3). Further function of the Tregs assessed by mean fluorescence intensity for CD120B did not reveal a main effect of time (p = 0.910) nor a training effect (p = 0.811).
<table>
<thead>
<tr>
<th></th>
<th>Pre-training</th>
<th>Post-training</th>
<th>Pre-training</th>
<th>Post-training</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD3+CD4+ (cells/ul)</td>
<td>293 (157)</td>
<td>300 (152)</td>
<td>320 (105)</td>
<td>289 (111)</td>
</tr>
<tr>
<td>CD3+CD8+ (cells/ul)</td>
<td>128 (102)</td>
<td>110 (140)</td>
<td>121 (41)</td>
<td>119 (45)</td>
</tr>
<tr>
<td>CD4+ (% cells)</td>
<td>58.4 (11.7)</td>
<td>58.0 (9.9)</td>
<td>55.7 (10.4)</td>
<td>54.7 (11.3)</td>
</tr>
<tr>
<td>CD8+ (% cells)</td>
<td>22.1 (9.7)</td>
<td>21.5 (10.1)</td>
<td>21.6 (8.1)</td>
<td>22.8 (8.7)</td>
</tr>
<tr>
<td>CD4+/CD8+ ratio</td>
<td>3.2 (1.7)</td>
<td>3.3 (1.8)</td>
<td>3.0 (1.5)</td>
<td>2.8 (1.3)</td>
</tr>
<tr>
<td>CD127-CD25+ (% cells)</td>
<td>3.3 (1.2)</td>
<td>2.8 (1.4)</td>
<td>2.7 (0.6)</td>
<td>3.4 (1.2)</td>
</tr>
<tr>
<td>CD127-foxP3+ (% cells)</td>
<td>1.2 (0.8)</td>
<td>1.2 (1.0)</td>
<td>1.0 (0.8)</td>
<td>1.0 (0.7)</td>
</tr>
</tbody>
</table>

Table 6.3. Absolute count and percentage of T cells in whole blood. Data displayed as mean (SD).

### 6.4.5 Functional inflammatory response

Half the lowest concentration of the standard curve was used for time points which presented with data that were out of range [417]. Following acute exercise during the 12th session, a significant effect of acute exercise was only present for IP-10 (p = 0.012) in the unstimulated condition, which demonstrated a 37.3% increase post-exercise when compared to pre-exercise (Figure 6.3). No significant interactions (p > 0.182) or main effects of time (pre- versus post-acute; p > 0.113) or training session (Session 1 vs Session 12; p > 0.255) were found for any other cytokines in the unstimulated condition.

When whole blood was stimulated with LPS to determine the response of cytokine production, no significant interaction effects (acute exercise response x training session; p > 0.147) or main effects of training (session 1 vs session 12; p > 0.274) were found. However, there were significant main effects in the acute exercise response (Figure 6.3), which were predominantly driven by the acute-exercise response in the 12th session. Of the pro-inflammatory cytokines, IL-1β, TNFα and IP-10 demonstrated an increase of 25% (p = .014), 55% (p = 0.045) and 140% (p = 0.022), respectively. The anti-inflammatory cytokine IL-10 also demonstrated an increase by 32% (p = 0.023) (Figure 6.3). However, despite the potential role of IL-8, IL-15 and MCP-1 on insulin resistance, none of these markers were affected following acute exercise nor exercise training (p > 0.165). Surprisingly, the increased production of IL-6 immediately post exercise in the 1st and 12th training session did not result in a significant difference (p = 0.063).
Figure 6.3. Mean (± SD) concentration of unstimulated and LPS-stimulated inflammatory cytokines pre- and post-exercise in the first and 12th session of training. *, post-exercise was greater than pre-exercise in the 12th session, p < 0.05. Closed circles represent first session; open circles represent 12th session.
6.5 Discussion

T2DM is characterized by an increased pro-inflammatory status [46], while exercise training is associated with an increase in anti-inflammatory status [418]. Acute exercise has also been shown to have profound effects on the inflammatory status [204], although paradoxically, acute exercise acutely increases pro-inflammatory cytokines. The purpose of this study was to determine whether an intensified training program, comprising 12 successive exercise training days can improve glucose tolerance in individuals with T2DM, and determine the associated changes in T-cell populations and their functional capacity in response to stimulation via a TLR4 ligand. The main findings were i) exercise training improved fructosamine concentrations, a short-term (2-3 week) marker of glycaemic control, although this was not associated with significant improvements in other markers of glucose tolerance or insulin sensitivity; ii) exercise training significantly reduced hsCRP, which was associated with a significant decrease in the pre-exercise concentration of AGP; iii) exercise training did not significantly alter T-cell populations, and resulted in a variable cytokine response. Given the profound immune-modulating effect of acute exercise, this study also sought to determine whether the acute response to exercise would be altered in response to the short-term training. The main findings were an increase in a number of the pro-inflammatory cytokines during the 12th session when compared to the first session, which appeared to be larger in magnitude than the rise in anti-inflammatory cytokines, which was contrary to our hypothesis.

Considering the short-term nature of the exercise protocol, the primary measure of glycaemic control adopted in the present study was a change in fructosamine concentration [412]. Fructosamine is an alternative measure of glycaemic control to HbA1c [53, 419], and provides a measure of glycosylated proteins (primarily albumin) which have shorter lifespans (1-3 weeks) than red blood cells (~17 weeks). In agreement with the stated hypothesis, there was a significant improvement in fructosamine concentration after the exercise training period. However, there were no significant improvements in fasting blood glucose, glucose AUC and insulin AUC, indices of insulin sensitivity (HOMA2-IR, HOMA2%beta, HOMA2%sensitivity & ISI composite); although all indices demonstrated favourable shifts in response to the exercise intervention. A number of studies have previously employed short-term training studies (≥7 consecutive days of exercise) in T2DM individuals and demonstrated improvements in measures of glycaemic control [40, 41, 188]. Similarly, participants with metabolic syndrome, but not T2DM improved glucose tolerance following 10 days of treadmill walking [420]. Fasting blood glucose was not improved which is consistent with previous work [40, 188] but not all studies, with O’Gorman and colleagues demonstrating significant improvements in fasting blood glucose and insulin sensitivity following 7 days of exercise, which was attributed to an increase in muscle GLUT4 content [41]. Of note, the
Cederholm index, a measure of peripheral insulin sensitivity [421], was associated with a moderate improvement \((d = 0.5)\) consistent with data indicating that exercise-based interventions are more likely to improve muscle sensitivity as opposed to hepatic insulin sensitivity [89].

The short-term exercise training was also effective in reducing the concentration of the acute phase reactant hsCRP, which has been well documented to be associated with T2DM [11, 12, 19] in addition to the development of cardiovascular disease [221]. This training-induced reduction highlights the lowered global level of inflammation and reduced cardiovascular risk despite the average concentration within this type 2 diabetic population remaining in the high-risk classification [10]. The reduction in CRP is also consistent with the improvements seen in longer term training studies lasting up to 10 months in individuals with [422] and without [43, 423] T2DM, further highlighting the effectiveness of just 12 days of exercise. Although IL-6 is correlated and a well-known precursor to the production of CRP [43], the reduction in CRP was not accompanied with a reduction in IL-6. This may be attributed to the lack of significant weight loss as previous work has reported an association between weight loss and a reduction in CRP and IL-6 [289].

AAGP is an acute phase glycoprotein that is primarily synthesised by hepatocytes in the liver and released as part of the acute phase response (peaking at a later time to CRP) which has been shown to positively associate with T2DM [396, 424-426]. Similarly to CRP, IL-6 in addition to IL-1β and TNFα have previously been reported to induce the expression of AAGP [425]. Hence, it is not surprising that AAGP was previously found to correlate with IL-6 and CRP in addition to all three markers predicting all-cause mortality [427]. Despite the lack of differences in resting concentration between the 1\(^{st}\) and 12\(^{th}\) exercise session, pre-exercise AAGP was lower in the 12\(^{th}\) exercise session when compared to the 1\(^{st}\) exercise session. Despite the immediate increase of AAGP after exercise on the 12\(^{th}\) session, the concentration was similar to both pre- and post-exercise concentration of the 1\(^{st}\) training session. As an acute phase reactant, the increase of AAGP is consistent with previous work which increased in concentration following a marathon [428]. However, training studies lasting between two to six months have previously found a reduction in this acute phase protein, which was associated with reduced visceral fat mass [429], a significant risk factor for the development of T2DM. Although body fat percentage and visceral adipose tissue did not change with exercise training, the lowered baseline AAGP concentration may be related to the outcome of CRP post training since both markers have previously been found to correlate with each other [427] and are associated with the risk of developing T2DM [46, 426]. It is recognized that AAGP was not measured at the same time as CRP which will limit interpretation of the relationship between
these two markers. However, the significant reduction in CRP demonstrates the beneficial impact exercise has on reducing systemic inflammation, which is also evident following long-term training of up to 10 months [43, 422, 423].

Unlike long-term training studies lasting between 3 and 12 months [44, 205, 430], we did not find any improvements in the inflammatory cytokines measured pre-exercise between the first and last exercise session. Together with hsCRP, the pro-inflammatory milieu has been well documented to be elevated in obese individuals with and without type diabetes; examples of these inflammatory cytokines include IL-1β, IL-6, IL-15, MCP-1, IP-10, TNFα [11-13, 19, 403, 431]. Previous work have documented significant improvements in low-grade inflammation following long-term exercise training (6 to 12 months), demonstrating the therapeutic effect of exercise by reduced pro-inflammatory cytokines including hsCRP, IL-1β, IL-6, IL-18, MCP-1, TNFα and increased anti-inflammatory cytokines including IL-10 and adiponectin [29, 44]. Despite these findings, previous research have also found no significant improvements in IL-6, IL-15, MCP-1, hsCRP and TNFα following 3 to 6 months of exercise training [205, 432]. However, when exercise was accompanied with weight loss and dietary modification, improvements were seen in pro-inflammatory markers compared to exercise alone, suggesting a reduction in fat mass may be more important than exercise alone [205].

Following the first training session, acute exercise resulted in increased IP-10. Although acute exercise did not affect any other inflammatory cytokines measured prior to and immediately after the first training session, pro- (IL-1β, IP-10, TNFα) and anti-inflammatory (IL-10) cytokines were increased following the 12th session. This was interesting to note as apart from IP-10, these markers were only affected following accumulation of the previous 12 exercise sessions.

Previous work has found that acute exercise leads to an inflammatory response releasing both pro- and anti-inflammatory cytokines such as IL-6, IL-1β, TNFα and IL-10 [433]. These cytokines are released as part of an acute phase response as a result of the exercise-induced suppression of the immune function increasing susceptibility to infection [434, 435]. During strenuous exercise, cytokines such as IL-6, IL-10, TNFα and IL-1β can be dramatically increased by up to ~100 fold, 27-fold, ~3-fold, ~2-fold, respectively [436]. The increase in these inflammatory cytokines is known to be affected following acute exercise which can be mediated by exercise intensity and duration [47, 278]. In the present study, when blood was collected post-exercise and cytokines were measured following LPS stimulation and 24 h incubation, we found that IL-6, IL-10, TNFα and IL-1β increased significantly following exercise in the 12th exercise session but not the 1st exercise session. The increase seen here
may have been a result of the accumulated training sessions leading to greater activation of the immune system and subsequently inducing greater functional production of these cytokines when stimulated with LPS. It is not surprising that IL-6 was increased which may be explained by the accumulated training period of 9 hours. Increased production of IL-6 following exercise has previously been suggested to be released from the muscle following contraction [437]. Additionally, the production of IL-6 may also act as an anti-inflammatory cytokine, stimulating the production of IL-10 [291], as well as inhibiting the production of TNFα [437]. The production of IL-10 is responsible for its suppressive ability in down-regulating pro-inflammatory cytokines [438]. The significance of IL-10 as an anti-inflammatory marker has previously been demonstrated such that IL-10 treatment prevented lipid-induced insulin resistance and protects skeletal muscle from obesity-associated macrophage infiltration and the detrimental effect cytokines have on insulin signaling and glucose metabolism [259]. Additionally, IL-10 also contributes to the expansion of Tregs which in turn amplifies the production of IL-10 to maintain immune homeostasis [292, 436]. Further production of Tregs has also been demonstrated to be induced by IL-10 under the presence of TGF-β [267]. Although an increase of IL-10 was seen post-exercise during the 12th session, no such changes were evident in measurement of TGF-β. Previous work has found that acute moderate-intensity acute exercise resulted in increased TGF-β concentration, but this was only evident 24h following exercise rather than immediately after exercise [439]. The lack of change in Tregs was consistent with no alterations found in CD3+CD4+ and CD3+CD8+ Tcells. The relationship between IL-10 and the therapeutic potential of Tregs has been shown to be associated with improved insulin sensitivity [258, 440]. Although a lack of change was seen within circulating Tregs in the present study, previous studies have demonstrated increased number of Tregs following acute exercise [309, 441] and training period [310, 311]. Consistent with a previous training study [311], we did not find any changes with TGF-β. However, in contrast with these findings, others have seen an improvement in TGF-β 24 h following exercise [439] which has an important role in inducing Tregs and its suppressive capacity [442]. It must also be stressed that only a limited number of exercise training related studies have previously been completed in humans [296, 310, 311, 443] and mice [26, 444]. To the author’s knowledge, this is the first study of intensified exercise training (12 consecutive days) in a type 2 diabetic population that has attempted to investigate the relationship of exercise training, Treg production, cytokine response and glucose tolerance. Little is known with pro-inflammatory marker IP-10 and its relationship with T2DM following exercise. Elevated IP-10 has previously been demonstrated in obese individuals with [445, 446] and without [447] T2DM compared to healthy individuals; and was positively correlated with risk markers of T2DM including BMI, fasting glucose fasting insulin, HbA1c and HOMA-IR, hsCRP and TNFα [448]. Immediately after the 12th exercise session, the
concentration of IP-10 increased by over 50% which suggests a greater pro-inflammatory environment. Although limited, our finding of increased IP-10 post-exercise is consistent with others [449] who found elevated IP-10 in post exercise in mice. Pro-inflammatory marker, RANTES (Regulated upon Activation Normal T cells Expressed and Secreted) has previously been found to positively correlate with IP-10 [447]. Furthermore, 3 months of exercise training was found to effectively reduce circulating levels of RANTES in obese humans, but the changes in IP-10 post-training could not be confirmed as the data was not available [447]. Recent work has also found that treadmill exercise reduced IP-10 in mice, which resulted in enhanced angiogenesis [450].

Although a number of positive outcomes were found in the present study demonstrating improved glycaemic control, aerobic capacity, anthropometry and inflammatory status, several limitations exists which could have further improved the understanding of the changes seen or lack thereof. An increased number of training sessions over a longer training period may result in greater improvements in glycaemic control as demonstrated by improved HbA1c following 2 months of training [451, 452] and improved glucose tolerance following 6 weeks of training [453]. Consequently, a longer training period of 12 weeks may lead to greater changes in Treg populations as previously documented [310, 311]. Additionally, the age and range and menopause may affect the inflammatory results as menopause is associated with increased concentration of CRP and TNFα [454]. A small sample size in this study may have obscured some significant outcomes. However, it is important to note that the calculated effect sizes were fairly small and may demonstrate the importance of adding some dietary modification into the intervention (beyond asking participants to maintain consistency). Finally, a control group would have strengthened the study.

In conclusion, the present study demonstrated that 12 days of treadmill exercise training in individuals with T2DM resulted in some improvements in glycaemic measures and reduced global inflammation. Future research should consider the inclusion of a larger sample size with the addition of suitable control group, appropriate dietary control, as well as a longer training period to determine if a greater training effect is evident. Additionally, extending the training period together with pre-diabetic and non-diabetic populations should be considered in the future.
Chapter 7  Thesis summary

The consequence of prolonged sedentary behaviour together with obesity leads to a state of chronic low grade inflammation which is associated with the development of T2DM [455]. Obesity is associated with an increased pro-inflammatory environment as a result of the infiltration of classically activated macrophages into the adipose tissue depots. These inflammatory responses are known to moderate the diurnal glycaemic excursions and expedite the development of T2DM, as well as increasing the risk of cardiovascular disease and all-cause mortality [57, 58, 61]. Exercise in contrast, is known to improve the glycaemic responses by increasing insulin sensitivity, and has been shown to affect the immune response both acutely and following exercise training. Hence, a better understanding regarding the role of exercise in managing glycaemic control and reducing chronic inflammation through exercise is important. The overarching aim of this dissertation was two-fold: i) To assess the role of exercise intensity on glycaemic control and the inflammatory response in individuals who were overweight/obese; ii) To determine the impact of 12 consecutive days of exercise on glycaemic control the inflammatory response both acutely following exercise and in response to the short-term training period.

Chapter Four investigated the role of exercise intensity on glucose tolerance and inflammatory markers involved in the IL-6 family. Comparison of exercise intensity was conducted using continuous moderate-intensity (CME; 65% \( \dot{V}O_{2\text{peak}} \)) exercise and high-intensity intermittent exercise (alternating bouts of high- and low-intensity exercise (HIIE); 100% and 50% \( \dot{V}O_{2\text{peak}} \), respectively). During each trial, glucose tolerance was assessed on three successive mornings at the same time of day whilst the inflammatory markers were measured before and immediately post-, 1 h post- and 25 h post-exercise (completed on second day). Our findings suggested that HIIE did not result in any additional benefit on glucose tolerance when compared to CME. This provided a unique contribution to the literature as previous work comparing between HIIE and CME have been limited by not matching for exercise duration and work output in overweight/obese males. With this in mind, appropriate interpretation of inflammatory data can be made comparing between the two exercise interventions. When the data were pooled, acute exercise (both interventions combined) was effective in reducing glucose AUC, although to a lesser extent than expected. However, this beneficial effect had disappeared 25 h post-exercise highlighting the importance of regular physical activity on managing glycaemic control. The release of muscle-derived IL-6 following acute exercise can promote glucose uptake [277, 295], with greater IL-6 concentration observed following HIIE than CME [364]. Although we did not find differences between interventions, IL-6 was
elevated immediately and 1 h post-exercise concurrent with a decrease in soluble receptors sgp130 and sIL-6R. Thus, the increase in IL-6/IL-6R ratio is consistent with ‘classical signalling’ which is postulated to contribute to improved glucose tolerance [369].

Adding to the findings in Chapter Four, Chapter Five assessed the response of acute exercise (CME vs HIIE) in T2DM related markers; matrix metalloproteinase (MMP)-2 and MMP-3, as well as OPN and adiponectin. These markers were assessed pre-exercise and immediately post-, 1 h post- and 25 h post-exercise. Glycaemic control under free-living conditions was also assessed for 72 h via the use of a continuous glucose monitoring system. The use of this system provided a unique insight into glucose concentrations between CME and HIIE measured in 5-minute intervals in overweight/obese males. Despite previous work demonstrating improvements in glucose control throughout the day following acute exercise [334] in T2DM, neither acute HIIE or CME was sufficient in improving glycaemic control measured throughout the oral glucose tolerance tests and glucose profile across the day. Measurement of the inflammatory markers revealed that MMP-2 and adiponectin were not affected with acute exercise. However, MMP-3 and OPN were acutely increased immediately post exercise which is consistent with a protective effect of T2DM, although this did not persist and returned to baseline levels the next day. Although these findings only apply to overweight individuals, further work is required to determine the response of these markers across different levels of impaired glucose tolerance, including individuals with T2DM.

Based on the findings from Chapters Four and Five in overweight/obese individuals at risk of T2DM, Chapter Six focused on the clinical impact exercise training may have on the combination of glycaemic control and immune-related changes. Specifically, individuals previously diagnosed with T2DM completed 12 consecutive days of treadmill exercise within a two-week period. Inflammatory cytokine (unstimulated and LPS-stimulated cytokine) responses to the first and last (12th) training session were assessed, in addition to measurement of regulatory T cells via flow cytometry. Markers of glycaemia as well as global inflammation marker, CRP were also assessed. Although glucose and insulin area under the curve did not improve post training, fructosamine, an alternative marker to HbA1c demonstrated a significant reduction highlighting improved glycaemic control following a short-term training period. Furthermore, a significant reduction in CRP was found following the training period. This is an important finding as CRP is a risk marker associated with the development of T2DM [11, 12] in addition to cardiovascular disease [221]. An increase in IP-10, TNFα, IL-1β and IL-10 were also observed following the last training session. These changes suggest an accumulative training effect leading to a greater inflammatory response although we expected a reduction in the pro-inflammatory cytokines and an increase in anti-inflammatory markers following training. The release of IL-10 in tandem with the pro-inflammatory cytokines is
necessary in order to maintain immune homeostasis which is known for its suppressive ability in dampening the pro-inflammatory response. Despite the cytokine response, exercise training did not induce any regulatory T cell responses in contrast to previous research which have reported increased number of Tregs acutely post-exercise [309] as well as following a training period [310, 311]. An increase in Tregs as reported in previous research would offer a sensible explanation to the increase in IL-10 post exercise [296]. Indeed, previous long term (≥ 12 weeks) training studies have reported improved glycaemic control (HbA1c) and reduced inflammation [29, 235, 284, 456, 457]. The findings in this chapter support a beneficial role of exercise training in reducing inflammation and improving glycaemic control which are detrimental to T2DM.

In summary, the results from this thesis indicated that an acute bout of HIIE in overweight/obese males does not confer additional benefits to CME on glycaemic control. Although an improvement in glucose AUC was evident immediately following exercise when data were pooled, the clinical significance of the improvement was relatively small and did not persist 25 h after exercise. This was also reflected in the lack of improvement in daily glycaemic control measured by continuous glucose monitoring system. Together with improved glucose AUC, we found a positive association between glucose AUC and IL-6 post exercise, accompanied by an increase in IL-6/IL-6R ratio. However, this effect was transient and returned to baseline 25 h post exercise. This trend was also consistent in the additional inflammatory markers related to T2DM, MMP-3 and OPN where its protective effect was acute and did not persist the following day. With this in mind, we investigated the role of short-term training in previously diagnosed type 2 diabetics to further determine the acute inflammatory response as well as the impact of exercise training. A significant increase in pro- and anti-inflammatory markers were evident following the last training session. Although the measurement of these cytokines was limited to only pre- and immediately post-exercise, it is plausible that the significant increase in IL-10 may have been crucial in dampening the pro-inflammatory response. Evidence has shown that Tregs are essential in the production of IL-10. Unfortunately, the increase in IL-10 was not concurrent with an increase in Tregs which may have explained for the increased IL-10 concentration. Although interpretation of the acute inflammatory response is limited, exercise training was indeed effective in reducing CRP, a global marker of inflammation which was also accompanied with a significant improvement in fructosamine. Interpretation of these findings were further limited by a restricted selection of inflammatory markers, where a composite score of a large number of related markers may be more informative. Furthermore, the inflammatory markers measured in this thesis were mainly of systemic origin rather than its tissue-specific source. Although systemic markers may present a ‘snapshot’ of the downstream inflammatory response, cytokine assessment from
adipose tissue and/or skeletal muscle may provide improved interpretation on the markers of interest to further understand the inflammatory response and its relationship with glycaemic control.

7.1 Future research directions

The mixed findings in glycaemic control in Chapter Four and Chapter Five pose further questions on the role acute exercise has on blood glucose management. These chapters provided clarity such that high-intensity intermittent exercise did not yield greater improvements in glycaemic control following acute exercise when compared to moderate-intensity exercise. To the author’s knowledge, these chapters were the first to compare between two exercise intensities that were matched for exercise duration and workload. However, a control group was not included which would have further strengthened the studies in comparing glucose tolerance acutely, as well as the glycaemic changes over the 72 h period. Whilst insulin and c-peptide were measured, limitations to understanding the complete picture of glucose metabolism was apparent as we did not measure a number of other substrates and hormones including free fatty acids, glucagon and epinephrine. Measurement of these analytes may provide greater clarity on the metabolic and hormonal balance where glucose tolerance was improved immediately post-exercise, but not 25 h following exercise. Additionally, frequent sampling of blood markers over a longer period of time (up to 4 hours) post exercise and additional methods such as isotope-labelled tracer technique may allow improved interpretation on the acute effects of exercise on glucose disposal.

Chapter Six investigated the effect of 12 days of consecutive training on glycaemic control and the inflammatory response following acute exercise and following the training period. Chronic inflammation and type 2 diabetes is an area of research that requires a greater understanding of how exercise may be beneficial in both reducing the level of inflammation together with the aim of improving glycaemic control. Chapter Six combined the measures of regulatory T cells, together with the inflammatory cytokine milieu and metabolic markers pre-and post-exercise to assess if either acute exercise and/or exercise training modulated these variables. Despite the measurement of these variables which attempt to clarify the relationship of exercise, inflammation and glycaemic control, the lack of a control group or another comparison group further limits the strength of the interpretation of the changes or lack of changes observed. Although the reduction in CRP and fructosamine were significant, there were no improvements in glucose tolerance post training which may have been the consequence of a small sample size. This should be a significant consideration for future research together with the addition of a control group to strengthen the study. Additionally, distinguishing differences of exercise intensity within such strict training period is required to
better understand the role of exercise intensity on glycaemic control and adaptations to the immune system. Although this thesis had primarily measured immune markers from blood, the assessment of the inflammatory response from key sources such as adipose tissue and skeletal muscle may lead to a better understanding on the regulation of the inflammatory response, both locally in the tissue and following a period of exercise. These differences may further disentangle the anti-inflammatory effect of exercise on adipose-tissue derived inflammation.

Bibliography


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Appendix A  Murdoch University Ethics Approval
A.1 Murdoch University Ethics Approval (Chapters 4 and 5)

Monday, 5 August 2013

Dr Timothy Fairchild
School of Psychology and Exercise Science
Murdoch University

Dear Timothy,

Project No. 2013/121
Project Title The effects of exercise intensity on immune function and glucose regulation

Thank you for addressing the conditions placed on the above application to the Murdoch University Human Research Ethics Committee. On behalf of the Committee, I am pleased to advise the application now has:

OUTRIGHT APPROVAL

Approval is granted on the understanding that research will be conducted according the standards of the National Statement on Ethical Conduct in Human Research (2007), the Australian Code for the Responsible Conduct of Research (2007) and Murdoch University policies at all times. You must also abide by the Human Research Ethics Committee’s standard conditions of approval (see attached). All reporting forms are available on the Research Ethics web-site.

I wish you every success for your research.

Please quote your ethics project number in all correspondence.

Kind Regards,

Dr. Erich von Dietze
Manager of Research Ethics

cc: Dr Jeremiah Pettifer and Aaron Raman
A.2 Murdoch University Ethics Approval
(Chapters 6)

Monday, 21 December 2015

Dr Timothy Fairchild
School of Psychology and Exercise Science
Murdoch University

Dear Timothy,

Project No. 2015/220
Project Title Moderate-intensity running vs cycling on glucose tolerance and immune function in type 2 diabetic individuals

Thank you for addressing the conditions placed on the above application to the Murdoch University Human Research Ethics Committee. On behalf of the Committee, I am pleased to advise the application now has:

OUTRIGHT APPROVAL

Approval is granted on the understanding that research will be conducted according the standards of the National Statement on Ethical Conduct In Human Research (2007), the Australian Code for the Responsible Conduct of Research (2007) and Murdoch University policies at all times. You must also abide by the Human Research Ethics Committee’s standard conditions of approval (see attached). All reporting forms are available on the Research Ethics and Integrity web-site.

I wish you every success for your research.

Please quote your ethics project number in all correspondence.

Kind Regards,

Dr. Erich von Dietze
Manager
Research Ethics and Integrity

cc: Dr Jeremiah Peiffer, Prof Gerard Hoyne, Dr Andrew Currie and Aaron Raman
Monday, 06 February 2017

Dr Timothy Fairchild
School of Psychology and Exercise Science
Murdoch University

Dear Timothy,

Project No. 2015/220
Project Title Moderate-intensity treadmill exercise on glucose tolerance and the immune function in type 2 diabetic individuals

AMENDMENT:  Removal of the cycling exercise group
Change of project title
Replacement of oral glucose tolerance test with blood sampling pre- and post-exercise

Your application for an amendment to the above project, received on 1/02/2017 was reviewed by the Murdoch University Human Research Ethics Committee and was;

APPROVED

Approval is granted on the understanding that research will be conducted according the standards of the National Statement on Ethical Conduct in Human Research (2007), the Australian Code for the Responsible Conduct of Research (2007) and Murdoch University policies at all times. You must also abide by the Human Research Ethics Committee’s standard conditions of approval. All reporting forms are available on the Research Ethics and Integrity web-site.

I wish you every success for your research.

Please quote your ethics project number in all correspondence.

Kind Regards,

Dr. Erich von Dietze
Manager
Research Ethics and Integrity

cc: Dr Jeremiah Peiffer, Prof Gerard Hoyne, Dr Andrew Currie and Aaron Raman
Appendix B Information Letters
The effects of exercise intensity on immune function and insulin sensitivity

We invite you to participate in a research study which aims to determine the effects of high-intensity exercise on glucose regulation (the control of blood sugar levels). Additionally, this study will seek to explore how the immune system is affected following an acute bout of exercise of varying exercise intensities. This study is part of my PhD in Exercise Science and is being supervised by Dr Timothy Fairchild and Dr Jeremiah Peiffer.

Nature and Purpose of the Study

This study aims to determine how exercise can improve the effectiveness of the hormone insulin (called insulin sensitivity). Insulin plays an important role in keeping blood sugar levels stable and in particular, the sugar known as glucose. We are particularly interested in how different levels of exercise intensity may change glucose levels and insulin sensitivity, and whether this relates to changes in the immune system.

Therefore the aim of this study is to investigate how different exercise intensities affect blood glucose levels, insulin sensitivity and markers of immune function.

If you consent to take part in this research study, it is important that you understand the purpose of the study and the procedures you will be asked to undertake. Please make sure you ask any questions you may have, and that all your questions have been answered to your satisfaction before you agree to participate.

What the Study will involve

Eligibility criteria: Since this project requires completion of an exercise bout and measurement of blood glucose and immune function, we are looking for a specific population group to participate in this study, namely those:
1. overweight male participants between 18 and 44 years old.
2. who have not been told they have Type 2 Diabetes.

You may be ineligible if you:
1. are taking any medication on a daily basis for long-term health reasons (such as Statins, blood pressure medication etc.)
2. have uncontrolled high blood pressure
3. have an acute (i.e. viral infection) or chronic (i.e. asthma [≥moderate persistent], cancer, arthritis, atherosclerosis) inflammatory disease.
4. are screened as ineligible from the Exercise and Sports Science screening tool.
5. do not have a waist circumference greater than 102cm

If you consent to participate in this study, you will be asked to attend the Murdoch University Exercise Physiology laboratory on seven separate occasions; the first visit will involve preliminary measurements and familiarisation to the procedures, whilst the other six visits will be part of the experimental trials; which will include completion of a 45-minute exercise bout during each trial. The exercise protocols we are interested in testing include a traditional exercise bout (constant intensity; CME) at 60% VO2peak and high-intensity intermittent exercise bouts (HIT) that consist of alternating high- and low-intensity exercise bouts for 60% @ 100% VO2peak and 24% @ 30% VO2peak. This exercise regime has gained recent popularity due to its proven effectiveness on certain health
parameters. We will be measuring the change in insulin sensitivity and immune function prior to and following the exercise bouts.

Specifically, the visits will include the following:

Visit 1: This visit will act as a baseline session in order to collect preliminary measurements and to familiarise yourself to the equipment, protocol and procedures related to the study. Any additional questions may be addressed at this stage. Screening questionnaires will be completed at this stage. Preliminary measurements include anthropometric assessment, body composition (DEXA scan), VO_{2peak} test (common fitness assessment), anaerobic power test, height and weight, and waist circumference.

Visit 2 to 4 (first trial): You will be asked to attend the laboratory prior to eating breakfast (overnight fast; >10 hours) and asked to remain seated while a Continuous Glucose Monitoring System (CGMS) is inserted under the skin into an area of subcutaneous fat in the abdominal area. The CGMS is the size of an iPod nano and will allow us to monitor glucose concentrations throughout the duration of each trial. Following insertion of the CGMS, an oral glucose tolerance test (OGTT) will be completed (described below). Before leaving the laboratory, you will be asked to record your food intake over the next 2 days and your activity levels monitored by a device called an accelerometer (similar to a pedometer). We will then provide you with an up-and-go drink and a muffin to eat about an hour after the visit.

The next morning (visit 3), you will again be asked to attend the laboratory prior to eating breakfast (overnight fast; >10 hours) and asked to remain seated while a pre-exercise venous blood sample (7mL; similar to giving blood at a pathology lab) is collected. The exercise intervention (i.e. HITT) will then be completed followed by a post-exercise 7mL venous blood sample. During exercise, you will breathe through a mouthpiece which is connected to the metabolic cart. The metabolic cart will measure total volume of air expired and the concentration of oxygen and carbon dioxide from expired breath. Heart rate and rating of perceived exertion will also be monitored continuously. Immediately after exercise, you will have a recovery period for an hour whilst remaining in the laboratory. Following the recovery period, a venous sample will be collected and an OGTT conducted. We will then provide you with an up-and-go drink and a muffin.

The next morning (Visit 4), you will again be asked to attend the laboratory prior to eating breakfast (overnight fast; >10 hours) and asked to remain seated while a venous blood sample is collected. An OGTT will then be completed. You will be provided with an up-and-go drink and a muffin. We will come and collect the CGMS unit the next day.

Visit 2 to 7 (second trial): These visits will be the same as those above (visits 2 to 4) - the only exception being that we perform the other exercise bout - one week later.

Further information of procedures:

VO_{2peak} test and anaerobic power test: These tests are used to measure fitness. VO_{2peak} determines the maximum amount of oxygen that your body can consume and this is typically measured during a bout of exercise. This test consists of cycling on a stationary bike at progressively difficult workloads whilst breathing through a mouthpiece connected to the metabolic cart. This test continues until volitional exhaustion. The highest oxygen uptake measured will then be deemed as VO_{2peak}. Heart rate will be continuously monitored throughout the test, and rating of perceived exertion (RPE; Borg scale) recorded.
every minute. Following the completion of the VO_{2max} test, you will then complete the anaerobic power test which consists of two 15-second efforts, interspersed with a 240-second recovery.

Oral Glucose Tolerance Test (OGTT): You will be asked to consume a drink containing 75g of glucose in 300mL of water. Finger-tip blood samples will be collected at the start of the OGTT and at 15, 30, 60, 90, and 120 min after the drink.

Anthropometric measurements: Height and weight will be measured using a stadiometer and weighing scale, respectively, and the Body Mass Index (BMI) calculated. Waist circumference will also be measured.

Body Composition Analysis: A Dual-Energy X-ray Absorptiometry (DEXA) scan will be completed to determine body composition. DEXA is a form of ionising radiation and is considered the gold-standard in body composition assessment. While ionising radiation presents with risks, the amount of ionising radiation is extremely low (0.15 microsieverts; similar to the amount of radiation received during a transatlantic flight). For example, a chest X-ray is 50 microsieverts (equivalent to 333 DEXA scans). Any potential risk is therefore negligible at doses this low.

Continuous Glucose Monitoring System (CGMS): The CGMS allows measurement of glucose values over a maximum period of 6 days. However, in this study we will only be using it over the three-day period (including the night of the third day). It is a common tool used in individuals with diabetes to track changes in glucose concentration over time. A finger prick test must be done at least twice daily to help calibrate the device throughout the day; we will ask you to perform one test each night, otherwise the researcher will be able to visit and perform this for you. The CGMS insertion guide will be provided to you along with this information letter.

Collection of blood: To determine the markers of the immune response, 7ml of blood will be collected from the antecubital vein (region of the arm in front of the elbow) on four occasions in visits 2 to 4 and visits 5 to 7. Fingers-prick blood samples from the OGTT will be used to measure concentrations of glucose and insulin.

Voluntary Participation and Withdrawal from the Study
Your participation in this study is entirely voluntary. You may withdraw at any time without discrimination or prejudice. All information is treated as confidential and no names or other details that might identify you will be used in any publication arising from the research. If you withdraw prior to completion of data collection, all information you have provided will be destroyed.

Benefits of the Study
Benefits of participation include a free fitness test (the gold-standard VO_{2max} test) and a body composition analysis by DEXA (gold-standard body composition analysis). In addition, we will provide you with a $50 giftcard to be used for meals throughout the duration of the study.

Possible Risks
It is possible that you may experience some discomfort during the session as a result of some of the tests such as the exercise or the blood sampling. You will be monitored closely.
during the study and you are free to withdraw at anytime during the study. There are only minor risks associated with the exercise protocol and blood sampling, although there will be some level of discomfort during the study. Some individuals may suffer light-headedness or fainting during the blood collection process, please inform the investigators if you have experienced this previously. Individuals may have bruising, numbness and pain which could last for a few days as a result of the blood collection from the finger. Insertion of the CGMS may cause mild initial discomfort but will be minimised by following safe practice as recommended by the manufacturers. High-intensity exercise will be a strenuous form of exercise which has been implemented in a plethora of previous research. Minimal risk factors include light-headedness, nausea and feeling breathless. You will be constantly monitored but please inform the researchers if you experience any feeling of great discomfort during the exercise conditions. It is important for you to understand that you can ask the investigator to stop the experiment at any stage without having to provide an explanation. All researchers in this study are trained (and current) in Senior First Aid.

If you have any questions about this project please feel free to contact myself Mr Raman (9360 1389; or A.Raman@murdoch.edu.au) or Dr Fairchild (9360 2059; or T.Fairchild@murdoch.edu.au) or Dr Peiffer (9360 7603; J.Peiffer@murdoch.edu.au). We will be happy to discuss with you any concerns you may have about this study.

Once we have analysed the information from this study we will publish the results of the study on the Murdoch University School of Chiropractic website: http://www.murdoch.edu.au/School-of-Psychology-and-Exercise-Science/Research/Exercise-Science-Research/Research-findings/. You can expect to receive this feedback within 12 months.

If you are willing to consent to participation in this study, please complete the Consent Form. Thank you for your assistance with this research project.

Sincerely,
Mr Aaron Raman

This study has been approved by the Murdoch University Human Research Ethics Committee (Approval 2013/121). If you have any reservation or complaint about the ethical conduct of this research, and wish to talk with an independent person, you may contact Murdoch University’s Research Ethics Office (Tel: 08 9360 6677 (for overseas studies, +61 8 9360 6677) or e-mail
Moderate-intensity treadmill exercise on glucose tolerance and immune function in type 2 diabetic individuals.

We invite you to participate in a research study which aims to assess the mode of exercise training on glucose regulation (the control of blood sugar levels) over a period of 2 weeks. Additionally, this study will seek to explore how the immune system is affected from treadmill of exercise. This study is part of any PhD in Exercise Science and is being supervised by Dr Timothy Fairchild and Dr Jeremiah Peiffer.

Nature and Purpose of the Study
This study aims to determine the effect of treadmill exercise over a 2-week training period and how it can improve glucose tolerance (how well the body disposes of glucose). Exercise is well known to be effective in managing type 2 diabetes by reducing fasting blood glucose as well as improving glucose tolerance. A result of improved blood glucose control leads to less reliance on insulin injections and/or glucose-lowering medication. A second part to this study is to understand how exercise training affects the immune system and if the exercise-induced increase in inflammation is related to glucose regulation.

If you consent to take part in this research study, it is important that you understand the purpose of the study and the procedures you will be asked to undertake. Please make sure you ask any questions you may have, and that all your questions have been answered to your satisfaction before you agree to participate.

What the Study will involve
Eligibility criteria: Since this project involves exercise training and measurement of blood glucose and immune function, we are looking for a specific population group to participate in this study, namely those:
1. Overweight or obese individuals between 18 and 60 years old.
2. Previously diagnosed with Type 2 Diabetes.

You may be ineligible if you:
1. Are unable to complete exercise or have a condition which may be aggravated by exercise.
2. Have had previous weight loss surgery
3. Have an acute (i.e. viral infection) or chronic (i.e. asthma [>moderate persistent], cancer, chronic rheumatoid arthritis, atherosclerosis) inflammatory disease.
4. Have a prior history of heart, lung, kidney, other endocrine disorders or liver disease
5. Have experienced recent weight loss of ≥4kg in previous month
6. Are pregnant
Sessions will be held at the Murdoch University Exercise Physiology laboratory on four occasions, in addition to twelve 45-minute training sessions held over a two week period. Exercise will consist of moderate-intensity exercise equivalent to 60-65% of your relative VO2peak test.

**Familiarisation session**

Your first visit to the laboratory will be a familiarisation session. In this session, baseline measurements will be completed which include anthropometric measurements, body composition (DEXA scan) analysis, blood pressure, an oral glucose tolerance test and a fitness test (VO2peak test; graded treadmill test).

This study has two components within the two-week period; an acute and a training phase. In the acute phase, we assess the mode of exercise following the first training session (acute) on glucose tolerance and immune function when compared to pre-exercise measurements. Prior to attending the first training session, you are asked to complete a 3-day food diary and physical activity monitoring with an Actigraph (tri-axial pedometer) provided. This will be repeated prior to the last training session to maintain similar lifestyle habits prior to blood collection. You are also asked to refrain from vigorous physical activity and alcohol in the 24 hours prior to the session.

**Acute phase:** The day before the first training session, you will arrive to the laboratory in a fasted state following placement of an intravenous catheter by a trained phlebotomist into the vein of your forearm. This allows us to collect blood samples at various time points throughout a glucose tolerance test. The blood samples will be used for the assessment of metabolic markers and immune function.

**Two-week training:** The remaining nine training sessions will be completed consecutively over the two-week period. 16-24th following completion of the final training session, an oral glucose tolerance test will be completed with blood samples collected. This will be repeated 48-72h following the last training session. The blood samples will be used for the assessment of metabolic markers and immune function. Anthropometric measurement, body composition analysis, an oral glucose tolerance test and a VO2peak test will also be repeated at the end of training period.

Throughout the training period, you will be asked to refrain from any other physical activity. Prior to the last training session, participants will be required to complete a 3-day food diary and physical activity monitoring as previously described.
Further information of procedures

VO2peak test: VO2peak determines the maximum amount of oxygen that the body can consume and this is typically measured during a bout of exercise. This test will consist of exercising at progressively difficult workloads on a treadmill whilst breathing through a mouthpiece connected to a metabolic cart. This test continues until volitional exhaustion.

Oral Glucose Tolerance Test (OGTT): You will be asked to consume a drink containing 75g of glucose in 300mL of water. Venous blood samples will be collected at the start of the OGTT and at 15-, 30-, 45-, 60-, 75-, 90-, 115- and 120-min thereafter.

Anthropometric measurements: Height and weight will be measured using a stadiometer and weighing scale, respectively, and the Body Mass Index (BMI) calculated. Waist circumference will also be measured.

Body Composition Analysis: A Dual-Energy X-ray Absorptiometry (DEXA) scan will be completed to determine body composition. DEXA is a form of ionising radiation and is considered the gold-standard in body composition assessment. While ionising radiation presents with risk, the amount of ionising radiation is extremely low (0.037 microsvert; similar to the amount of radiation received during a transatlantic flight).

Collection of blood: To determine the markers of the immune function, blood will be collected via an intravenous catheter from the antecubital vein (region of the arm in front of the elbow) before and after the two-week training period (within 16-24h of final session). The post-training blood samples will also be repeated 48-72h following the last training session. Blood samples will also be collected during the oral glucose tolerance tests for assessment of glucose and insulin.

Voluntary Participation and Withdrawal from the Study
Your participation in this study is entirely voluntary. You may withdraw at any time without discrimination or prejudice. All information is treated as confidential and no names or other details that might identify you will be used in any publication arising from the research. If you withdraw prior to completion of data collection, all information you have provided will be destroyed.

Benefits of the Study
Benefits of participation include a free fitness test (the gold-standard VO2max test), body composition analysis by DEXA (gold-standard body composition analysis) and a two-week supervised training program by an exercise physiologist.
Possible Risks

It is possible that you may experience some discomfort during the session as a result of some of the tasks such as the exercise or the blood sampling.

Some individuals may suffer light-headedness or fainting during the blood collection process, please inform the investigators if you have experienced this previously. Individuals may have bruising, numbness and pain which could last for a few days as a result of the blood collection. Minimal risk factors include light-headedness, nausea and feeling breathless. You will be constantly monitored but please inform the researchers if you experience any feeling of great discomfort during the exercise conditions. All researchers in this study are trained (and current) in Senior First Aid.

If you have any questions about this project please feel free to contact myself, Mr Raman (A.Raman@murdoch.edu.au) or Dr Fairchild (9360 2959; or T.Fairchild@murdoch.edu.au) We will be happy to discuss with you any concerns you may have about this study.

Once we have analysed the information from this study we will publish the results of the study on the Murdoch University School of Psychology and Exercise Science website.

If you are willing to consent to participation in this study, please complete the Consent Form. Thank you for your assistance with this research project.

Sincerely,

Mr Aaron Raman

This study has been approved by the Murdoch University Human Research Ethics Committee (Approval 2015/220). If you have any reservation or complaint about the ethical conduct of this research, and wish to talk with an independent person, you may contact Murdoch University’s Research Ethics Office (Tel. 08 9360 6677 (for overseas studies, +61 8 9360 6677) or e-mail ethics@murdoch.edu.au). Any issues you raise will be treated in confidence and investigated fully, and you will be informed of the outcome.
Appendix C Adult Pre-Exercise Screening Tool

ADULT PRE-EXERCISE SCREENING TOOL

This screening tool does not provide advice on a particular matter, nor does it substitute for advice from an appropriately qualified medical professional. No warranty of safety should result from its use. The screening system in no way guarantees against injury or death. No responsibility or liability whatsoever can be accepted by Exercise and Sports Science Australia, Fitness Australia or Sports Medicine Australia for any loss, damage or injury that may arise from any person acting on any statement or information contained in this tool.

Name: ____________________________
Date of Birth: ____________________    Male ☐    Female ☐    Date: ____________

STAGE 1 (COMPULSORY)

AIM: to identify those individuals with a known disease, or signs or symptoms of disease, who may be at a higher risk of an adverse event during physical activity/exercise. This stage is self-administered and self-evaluated.

Please circle response

1. Has your doctor ever told you that you have a heart condition or have you ever suffered a stroke?  Yes ☐ No ☐

2. Do you ever experience unexplained pains in your chest at rest or during physical activity/exercise?  Yes ☐ No ☐

3. Do you ever feel faint or have spells of dizziness during physical activity/exercise that causes you to lose balance?  Yes ☐ No ☐

4. Have you had an asthma attack requiring immediate medical attention at any time over the last 12 months?  Yes ☐ No ☐

5. If you have diabetes (type 1 or type 2) have you had trouble controlling your blood glucose in the last 3 months?  Yes ☐ No ☐

6. Do you have any diagnosed muscle, bone or joint problems that you have been told could be made worse by participating in physical activity/exercise?  Yes ☐ No ☐

7. Do you have any other medical condition(s) that may make it dangerous for you to participate in physical activity/exercise?  Yes ☐ No ☐

IF YOU ANSWERED YES to any of the 7 questions, please seek guidance from your GP or appropriate allied health professional prior to undertaking physical activity/exercise.

IF YOU ANSWERED ‘NO’ to all of the 7 questions, and you have no other concerns about your health, you may proceed to undertake light-moderate intensity physical activity/exercise.

I believe that to the best of my knowledge, all of the information I have supplied within this tool is correct.
Signature ____________________________ Date ____________

ESSA: Fitness Australia: Sports Medicine Australia:
<table>
<thead>
<tr>
<th>INTENSITY CATEGORY</th>
<th>HEART RATE MEASURES</th>
<th>PERCEIVED EXERTION MEASURES</th>
<th>DESCRIPTIVE MEASURES</th>
</tr>
</thead>
<tbody>
<tr>
<td>SEDENTARY</td>
<td>&lt; 40% HRmax</td>
<td>Very, very light RPE&lt; 1</td>
<td>• Activities that usually involve sitting or lying and that have little additional movement and a low energy requirement</td>
</tr>
<tr>
<td>LIGHT</td>
<td>40 to &lt;55% HRmax</td>
<td>Very light to light RPE 1-2</td>
<td>• An aerobic activity that does not cause a noticeable change in breathing rate</td>
</tr>
<tr>
<td>MODERATE</td>
<td>55 to &lt;70% HRmax</td>
<td>Moderate to somewhat hard RPE 3-4</td>
<td>• An aerobic activity that is able to be conducted whilst maintaining a conversation uninterrupted</td>
</tr>
<tr>
<td>VIGOROUS</td>
<td>70 to &lt;90% HRmax</td>
<td>Hard RPE 5-6</td>
<td>• An aerobic activity in which a conversation generally cannot be maintained uninterrupted</td>
</tr>
<tr>
<td>HIGH</td>
<td>≥ 90% HRmax</td>
<td>Very hard RPE ≥ 7</td>
<td>• An intensity that generally cannot be sustained for longer than about 10 minutes</td>
</tr>
</tbody>
</table>

*RPE* = Borg's Rating of Perceived Exertion (RPE) scale, category scale 0-10
ADULT PRE-EXERCISE SCREENING TOOL

STAGE 2 (OPTIONAL)

Name: ____________________________
Date of Birth: ______________________
Date: ______________________________

AIM: To identify those individuals with risk factors or other conditions to assist with appropriate exercise prescription. This stage is to be administered by a qualified exercise professional.

1. Age: ____________________________
   Gender: __________________________
   ≥ 45yrs Males or ≥ 55yrs Females +1 risk factor

2. Family history of heart disease (e.g., stroke, heart attack)
   Relative: __________________________
   Age: _____________________________
   If male < 55yrs = +1 risk factor
   If female < 65yrs = +1 risk factor
   Maximum of 1 risk factor for this question

3. Do you smoke cigarettes on a daily or weekly basis or have you quit smoking in the last 6 months? Yes: ____________ No: ____________
   If currently smoking, how many per day or week? ____________
   If yes, (smoke regularly or given up within the past 6 months): +1 risk factor

4. Describe your current physical activity/exercise levels:
   Frequency: __________________________
   Sedentary: ____________
   Light: ____________
   Moderate: ____________
   Vigorous: ____________
   If physical activity level < 150 min/week = +1 risk factor
   If physical activity level ≥ 150 min/week = -1 risk factor
   (vigorous physical activity/exercise weighted x 2)

5. Please state your height (cm): ____________ weight (kg): ____________
   BMI = ____________
   BMI ≥ 30 kg/m² = +1 risk factor

6. Have you been told that you have high blood pressure? Yes: ____________ No: ____________
   If yes, = +1 risk factor

7. Have you been told that you have high cholesterol? Yes: ____________ No: ____________
   If yes, = +1 risk factor

8. Have you been told that you have high blood sugar? Yes: ____________ No: ____________
   If yes, = +1 risk factor

Note: Refer over page for risk stratification.

STAGE 2 Total Risk Factors = ____________________________
9. Have you spent time in hospital (including day admission) for any medical condition/fatigue during the last 12 months? Yes No
   if yes, provide details

10. Are you currently taking a prescribed medication(s) for any medical condition(s)? Yes No
    If yes, what is the medical condition(s)?

11. Are you pregnant or have you given birth within the last 12 months? Yes No
    If yes, provide details. I am ___ months pregnant or postnatal (circle).

12. Do you have any muscle, bone or joint pain or soreness that is made worse by particular types of activity? Yes No
    If yes, provide details

---

**STAGE 3 (OPTIONAL)**

Aim: To obtain pre-exercise baseline measurements of other recognised cardiovascular and metabolic risk factors. This stage is to be administered by a qualified exercise professional. (Measures 1, 2 & 3 – minimum qualification, Certificate III in Fitness; Measures 4 and 5 minimum level, Exercise Physiologist*).

**RESULTS**

<table>
<thead>
<tr>
<th>Measure</th>
<th>Risk Factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. BMI (kg/m²)</td>
<td>BMI ≥ 30 kg/m² = +1 risk factor</td>
</tr>
<tr>
<td>2. Waist girth (cm)</td>
<td>Waist &gt; 94 cm for men and &gt; 80 cm for women = +1 risk factor</td>
</tr>
<tr>
<td>3. Resting BP (mmHg)</td>
<td>SBP ≥ 140 mmHg or DBP ≥ 90 mmHg = +1 risk factor</td>
</tr>
<tr>
<td>4. Fasting lipid profile*</td>
<td>Total cholesterol ≥ 5.20 mmol/L = +1 risk factor</td>
</tr>
<tr>
<td></td>
<td>HDL cholesterol &gt;1.55 mmol/L = -1 risk factor</td>
</tr>
<tr>
<td></td>
<td>HDL cholesterol &lt;1.00 mmol/L = +1 risk factor</td>
</tr>
<tr>
<td></td>
<td>Triglycerides ≥ 1.70 mmol/L = +1 risk factor</td>
</tr>
<tr>
<td></td>
<td>LDL cholesterol ≥ 3.40 mmol/L = +1 risk factor</td>
</tr>
<tr>
<td>5. Fasting blood glucose*</td>
<td>Fasting glucose ≥ 5.50 mmol/L = +1 risk factor</td>
</tr>
</tbody>
</table>

**RISK STRATIFICATION**

Total stage 2 or Total stage 3 Plus stage 2 (Q1 – Q4)

- > 2 RISK FACTORS – MODERATE RISK CLIENTS
  - Individuals at moderate risk may participate in aerobic physical activity/exercise at a light or moderate intensity (refer to the exercise intensity table on page 2)

- < 2 RISK FACTORS – LOW RISK CLIENTS
  - Individuals at low risk may participate in aerobic physical activity/exercise up to a vigorous or high intensity

*Note: If stage 3 is completed, identify risk factors from stages 1 (Q1– Q4) and stage 3 should be combined to reduce risk. If there are extreme or multiple risk factors, the exercise professional should use professional judgement to decide whether further medical advice is required.
Appendix D 24-h Food Diary

24-hour Dietary Intake Questionnaire
Individual Intake Form

<table>
<thead>
<tr>
<th>Quick List of Food Items</th>
<th>Column 1</th>
<th>Column 2</th>
<th>Column 3</th>
<th>Column 4</th>
<th>Code/role only</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Time</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B. Occasion</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A. Food/Drink and Additions</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B. Description of Food/Drink and Ingredient</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>How much of this (FOOD) did you actually (eat/drink)?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Where did you obtain the (FOOD)?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Food code</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amount</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>


Appendix E Borg Scale

BORG SCALE
Rating of Perceived Exertion

6
7  Very very light
8
9  Very light
10
11  Fairly light
12
13  Somewhat Hard
14
15  Hard
16
17  Very hard
18
19  Very very hard
20  Maximal exertion
Appendix F CONSORT Diagram

CONSORT 2010 Flow Diagram

Enrollment

Assessed for eligibility (n=18)
- Excluded (n=5)
  - Not meeting inclusion criteria (n=4)
  - Declined to participate (n=1)

Allocated to intervention (n=13)

Lost to follow-up (give reasons) (n=0)
Discontinued intervention (give reasons) (n=0)

Analysed (n=13) – Glucose markers
  - Excluded from analysis (n=0)
Analysed (n=9) – Regulatory T cells
  - Excluded from analysis (missed samples) (n=4)