Strategies to induce an inflammatory response: a focus on alternatives for people restricted to engage in lower-body exercise

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Strategies to induce an inflammatory response; a focus on alternatives for people restricted to engage in lower-body exercise

Sven Piersson Hoekstra

A Doctoral Thesis

Submitted in partial fulfilment for the requirements for the award of Doctor of Philosophy of Loughborough University

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Abstract

Introduction Chronic low-grade inflammation is increasingly recognised as a risk factor for non-communicable diseases such as type 2 diabetes mellitus and cardiovascular disease. Regular exercise has a protective effect against these type of diseases, possibly partially resulting from the acute inflammatory response and the subsequent anti-inflammatory milieu created following each bout of exercise. This acute response is characterised by an increased production of interleukin (IL)-6, heat shock protein 72 (Hsp72), and anti-inflammatory cytokines such as IL-1ra and IL-10. However, engaging in exercise of sufficient volume to induce an acute inflammatory response may not be feasible for those who are restricted to be physically active. In this thesis, factors that may influence the acute inflammatory response to exercise are investigated to inform strategies to reduce chronic low-grade inflammation in people with a low physical capacity. Furthermore, the efficacy of 2 such strategies, namely upper-body high-intensity interval training (HIIT) and hot water immersion (HWI), to induce an acute inflammatory response is investigated. Moreover, the effect of a chronic HWI intervention on the inflammatory profile and metabolic markers at rest is assessed. Methods In Chapters 2 and 3, factors that may influence the acute inflammatory response to exercise are investigated. The impact of chronic, modality-specific training adaptations and active muscle mass is investigated in individuals chronically trained in either upper- or lower-body exercise, while the role of autonomic function is studied in wheelchair athletes taking part in a wheelchair half-marathon. Thereafter, studies exploring the efficacy of relatively novel health promoting strategies to improve the inflammatory profile are presented. The acute increase in IL-6 and IL-1ra concentrations following upper-body HIIT is compared with moderate-intensity upper-body exercise in Chapter 4. In Chapters 5 and 6, the acute and chronic effects of HWI on inflammatory and metabolic markers in sedentary overweight males are studied. Finally, an in-vitro model is used to gain more insight in the potential of temperature elevations to induce an acute inflammatory response in monocytes (Chapter 7). The main inflammatory markers investigated in the present thesis are IL-6, intracellular Hsp72 in monocytes, extracellular Hsp72 and the distribution of monocyte subsets. Alongside the inflammatory markers, perceptual responses are collected to help inform the implementation of the studied
interventions. **Results** Chronic modality-specific training adaptations, the reduced active muscle mass and autonomic dysfunction have all shown to attenuate the acute inflammatory response following upper-body exercise. Although HIIT did not result in a larger acute increase in plasma IL-6 and IL-1ra concentrations, it was more time efficient and perceived as more enjoyable than moderate-intensity continuous exercise. A single HWI session induced an acute elevation in plasma IL-6 concentration, as well as a shift in the monocyte subset distribution. However, this was not accompanied by an increase in iHsp72 expression. A higher core temperature than observed in Chapter 5 (38.7±0.4°C) may be needed to increase iHsp72 expression; which was supported by the results of Chapter 7, where incubation of whole blood at 40°C but not 38.5°C increased iHsp72 expression in monocytes. A chronic 2-week HWI intervention reduced fasting glucose and insulin as well as eHsp72 concentrations at rest. No change in resting IL-6 or iHsp72 was observed following the intervention.

**Conclusion** The observation that a range of factors can reduce the potential of exercise to induce an acute inflammatory response in people restricted to engage in (lower-body) exercise may warrant additional health promoting strategies to reduce chronic low-grade inflammation in these individuals. The results of the present thesis support the potential of body temperature manipulations to be such a strategy. The lowering of fasting glucose and insulin, as well as eHsp72 following a relatively short-term chronic HWI intervention suggest that passively increasing body temperature may be a viable tool to improve the inflammatory profile and metabolic health in people restricted to be physically active.

*Keywords: Upper-body exercise – Hot water immersion – Chronic low-grade inflammation – Interleukin-6 – Heat shock protein 72 - Monocytes*
Publications

Journal articles:


Conference proceedings:


Invited presentations:


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<tr>
<td>BLa</td>
<td>Blood lactate concentration</td>
</tr>
<tr>
<td>BMI</td>
<td>Body mass index</td>
</tr>
<tr>
<td>CAD</td>
<td>Moderate-intensity exercise with change in cadence</td>
</tr>
<tr>
<td>CD</td>
<td>Cluster of differentiation</td>
</tr>
<tr>
<td>CI</td>
<td>Confidence interval</td>
</tr>
<tr>
<td>CON</td>
<td>Moderate-intensity continuous exercise</td>
</tr>
<tr>
<td>CRP</td>
<td>C-reactive protein</td>
</tr>
<tr>
<td>CSCl</td>
<td>Cervical spinal cord injury</td>
</tr>
<tr>
<td>CV</td>
<td>Coefficient of variation</td>
</tr>
<tr>
<td>CVD</td>
<td>Cardiovascular disease</td>
</tr>
<tr>
<td>EDTA</td>
<td>Ethylenediaminetetraacetic acid</td>
</tr>
<tr>
<td>eHsp72</td>
<td>Extracellular heat shock protein 72</td>
</tr>
<tr>
<td>ELISA</td>
<td>Enzyme-linked immunosorbent assay</td>
</tr>
<tr>
<td>eNOS</td>
<td>Endothelial nitric oxide synthase</td>
</tr>
<tr>
<td>FAS</td>
<td>Felt arousal scale</td>
</tr>
<tr>
<td>FS</td>
<td>Feeling scale</td>
</tr>
<tr>
<td>GMFI</td>
<td>Geometric mean fluorescence intensity</td>
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<tr>
<td>GXT</td>
<td>Graded exercise test</td>
</tr>
<tr>
<td>Hb</td>
<td>Haemoglobin</td>
</tr>
<tr>
<td>Hct</td>
<td>Haematocrit</td>
</tr>
<tr>
<td>HSE</td>
<td>Heat shock element</td>
</tr>
<tr>
<td>HSF-1</td>
<td>Heat shock factor-1</td>
</tr>
<tr>
<td>HSP</td>
<td>Heat shock protein</td>
</tr>
<tr>
<td>HWI</td>
<td>Hot water immersion</td>
</tr>
<tr>
<td>iHsp72</td>
<td>Intracellular heat shock protein 72</td>
</tr>
<tr>
<td>IL</td>
<td>Interleukin</td>
</tr>
<tr>
<td>IL-1ra</td>
<td>Interleukin-1 receptor antagonist</td>
</tr>
<tr>
<td>IL-6R</td>
<td>IL-6 receptor</td>
</tr>
<tr>
<td>IRS-1</td>
<td>Insulin receptor substrate-1</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
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<tr>
<td>--------------</td>
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<tr>
<td>JNK</td>
<td>C-Jun n-terminal kinase</td>
</tr>
<tr>
<td>LPS</td>
<td>lipopolysaccharide</td>
</tr>
<tr>
<td>LTan</td>
<td>Anaerobic lactate threshold</td>
</tr>
<tr>
<td>NF-κB</td>
<td>Nuclear factor kappa B</td>
</tr>
<tr>
<td>NO</td>
<td>Nitric oxide</td>
</tr>
<tr>
<td>NON-CSCI</td>
<td>Non-cervical spinal cord injured</td>
</tr>
<tr>
<td>PACES</td>
<td>Physical activity enjoyment scale</td>
</tr>
<tr>
<td>PBMC</td>
<td>peripheral blood mononuclear cell</td>
</tr>
<tr>
<td>PBS</td>
<td>Phosphate buffered saline</td>
</tr>
<tr>
<td>PO</td>
<td>Power output</td>
</tr>
<tr>
<td>PO_{peak}</td>
<td>Peak power output</td>
</tr>
<tr>
<td>Rc</td>
<td>Regression coefficient</td>
</tr>
<tr>
<td>RPE</td>
<td>Rating of perceived exertion</td>
</tr>
<tr>
<td>RPE_{C}</td>
<td>Central rating of perceived exertion</td>
</tr>
<tr>
<td>RPE_{L}</td>
<td>Local rating of perceived exertion</td>
</tr>
<tr>
<td>RPE_{O}</td>
<td>Overall rating of perceived exertion</td>
</tr>
<tr>
<td>SCI</td>
<td>Spinal cord injury</td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>sFS</td>
<td>Session feeling scale</td>
</tr>
<tr>
<td>sRPE</td>
<td>Session rating of perceived exertion</td>
</tr>
<tr>
<td>T2DM</td>
<td>Type 2 diabetes mellitus</td>
</tr>
<tr>
<td>TC</td>
<td>Thermal comfort</td>
</tr>
<tr>
<td>T_{core}</td>
<td>Core temperature</td>
</tr>
<tr>
<td>T_{re}</td>
<td>Rectal temperature</td>
</tr>
<tr>
<td>TLR</td>
<td>Toll-like receptor</td>
</tr>
<tr>
<td>TNF-α</td>
<td>Tumour necrosis factor-α</td>
</tr>
<tr>
<td>TS</td>
<td>Thermal sensation</td>
</tr>
<tr>
<td>T_{skin}</td>
<td>Skin temperature</td>
</tr>
<tr>
<td>VO_{2}</td>
<td>Oxygen uptake</td>
</tr>
<tr>
<td>VO_{2peak}</td>
<td>Peak oxygen uptake</td>
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</table>
Synopsis

Physical inactivity and a surplus of body fat can lead to elevated resting concentrations of circulating pro-inflammatory proteins; a state called *chronic low-grade inflammation* (Alves et al., 2016). Chronic low-grade inflammation is increasingly recognised in the aetiology of a range of chronic diseases, such as type 2 diabetes mellitus (T2DM) and cardiovascular disease (CVD), and may therefore serve as a target in the prevention or treatment of these diseases (Dandona et al., 2004). While the prevalence of non-communicable diseases is high in the general population (World Health Organisation, 2018), wheelchair users, and those with a spinal cord injury (SCI) in particular, are at an even further increased risk (Bauman and Spungen, 2008). This may be related to the injury itself, but could also stem from a physically inactive lifestyle, as people with SCI are generally less physically active compared with able-bodied individuals (Martin Ginis et al., 2010).

While regular exercise training can indeed be effective in reducing chronic low-grade inflammation (Petersen and Pedersen, 2005), this may not necessarily be the case for people with SCI. For instance, the reduced muscle mass involved in upper-body exercise and the autonomic dysfunction present in some individuals with SCI may attenuate the acute inflammatory response to exercise (Paulson et al., 2013), which is believed to be an important driver of the anti-inflammatory effects of exercise (Petersen and Pedersen, 2005). Furthermore, as members of this population can be restricted to engage in sufficient volumes of exercise due to their relatively low physical capacity, alternatives to exercise may serve useful to enhance cardiometabolic health in this population. Moreover, as a limited physical capacity is present in a variety of other populations, such as the elderly or chronically ill, the implications of such alternatives go beyond people with SCI only.

To inform the creation of strategies that could reduce chronic low-grade inflammation in populations restricted to engage in (lower-body) exercise, factors that may influence the acute inflammatory response to exercise are investigated. Moreover, alternative health promoting strategies are explored for their efficacy to induce an acute inflammatory response and improve the
inflammatory and metabolic profile at rest. The structure of the thesis is described below, and a schematic overview is provided in Fig. S1.

Structure of the thesis

Following a general introduction in Chapter 1, the results of the conducted studies are presented in 6 experimental chapters. Chapter 2 investigates the influence of active muscle mass and chronic training adaptations on the acute inflammatory response to exercise by assessing acute physiological and inflammatory responses to upper- and lower-body exercise in people chronically trained in either of these 2 modalities. Focussing on specific disability-related influences on the acute inflammatory response in individuals with SCI, Chapter 3 investigates the impact of autonomic function on the acute inflammatory response to a wheelchair half-marathon. In the subsequent chapters, the efficacy of alternative or additional health promoting strategies suitable for people with SCI and other populations restricted to engage in (lower-body) exercise are explored. In Chapter 4, the acute inflammatory response to a relatively novel and time-efficient form of upper-body exercise (i.e. high-intensity interval training (HIIT)) is assessed in comparison with more traditional moderate-intensity continuous exercise. Focussing on passive health promoting strategies, Chapter 5 assesses the acute inflammatory response to a session of hot water immersion (HWI) in sedentary, overweight males, while Chapter 6 investigates the effects of a chronic HWI intervention on resting inflammatory and metabolic markers. Chapter 7 uses an ex-vivo model to further explore the influence of temperature and Hsp72 on the acute inflammatory response in monocytes. Finally, the implications of the findings resulting from this thesis are discussed in Chapter 8.
Factors influencing the acute inflammatory response to exercise

Chapter 2
To investigate the influence of chronic modality-specific training adaptation on the acute inflammatory response to upper-and lower-body exercise in individuals trained in either modality.

Chapter 3
To investigate the influence of autonomic function on the acute inflammatory response to a wheelchair half-marathon in recreational wheelchair athletes.

Chapter 4
To compare the perceptual and inflammatory responses between upper-body HIIT and moderate intensity exercise in recreationally active males.

Chapter 5
To investigate the acute inflammatory response to a HWI session in overweight, sedentary males.

Chapter 6
To investigate the effect of a 2-week HWI intervention on resting metabolic and inflammatory markers in overweight, sedentary males.

Chapter 7
To investigate the influence of temperature and eHsp72 on the expression of iHsp72 and IL-6 in monocytes, using an ex-vivo model.

Fig. S1 Overview of the main aims of the chapters presented in this thesis. Abbreviations: HIIT = high-intensity interval training; eHsp72 = extracellular heat shock protein 72; IL-6 = interleukin-6; iHsp72 = intracellular heat shock protein 72; HWI = hot water immersion
General introduction
It is estimated that only 39% of United Kingdom citizens meet the physical activity guidelines and that 26% are obese (British Heart Foundation, 2017). Similar statistics are reported for other countries in the Western world and increasingly so for developing countries (Wild et al., 2004). Physical inactivity and a surplus of body fat are independent risk factors for chronic diseases such as T2DM, CVD and some forms of cancer (Wilmot et al., 2012). There is increasing evidence to suggest that the link between physical inactivity, obesity and the onset of the aforementioned chronic diseases is of inflammatory nature (Alves et al., 2016). Many studies have now indicated that a state of chronic low-grade inflammation is associated with T2DM and CVD (Henstridge et al., 2014b; Ridker, 2003). Moreover, emerging evidence supports a direct impact of inflammation in the aetiology of those diseases (Minihane et al., 2015). Therefore, chronic low-grade inflammation is a target for the prevention and treatment of these non-communicable diseases.

1.1 Chronic low-grade inflammation and chronic disease

Although traditionally mainly acknowledged as the system to defend individuals against invading pathogens, it is now widely recognised that the immune system also plays an important role in the maintenance of cardiometabolic health (Ridker, 2003). The immune system can be broadly divided into the innate and adaptive arm. The innate part of the immune system responds quickly and non-specifically to invading pathogens, particles it recognises as “non-self” and more general stressors such as hypoxia or starvation. It consists, among other things, of different types of leukocytes, with monocytes, macrophages, neutrophils, dendritic cells and natural killer cells representing the largest proportion. Besides their role to digest and kill micro-organisms, these immune cells also secrete cytokines to activate other immune cells and tissue in response to the different stressors and invading pathogens (Akira et al., 2006). Hence, the inflammatory response induced by the innate immune system is an evolutionary preserved response to protect individuals from invading pathogens and factors that can perturb homeostasis. Although this acute inflammatory response can clearly be regarded as beneficial to the individual, chronic activation of the immune system (i.e. chronic low-grade inflammation) might have a detrimental influence on health (Dandona et al., 2004). For instance, chronically elevated plasma concentrations of pro-inflammatory cytokines
can impair insulin sensitivity by inhibiting phosphorylation of the insulin receptor substrate-1 (IRS-1) (Hotamisligil, 2003) and vascular integrity by facilitating the filtration of macrophages through the vascular wall to form atherosclerotic plaques (Baker et al., 2011).

Since the recognition of chronic-low grade inflammation as a risk factor for chronic diseases, a range of possible markers to best describe and target this state have emerged. Elevated basal plasma concentrations of interleukin (IL)-6, tumour necrosis factor alpha (TNF-α) and C-reactive protein (CRP) are commonly used in this respect (Dandona et al., 2004). In addition, high expression of Toll-like receptors 2 and 4 (TLR2 and TLR4) on monocytes (Flynn et al., 2006) and a relatively large proportion of intermediate and non-classical monocytes present in the circulation are associated with this inflammatory state (Wong et al., 2012). Lastly, there is a growing body of evidence on the importance of heat shock protein (HSP) in the aetiology T2DM and CVD (Henstridge et al., 2014b; Hooper et al., 2014).

Cytokines

Interleukin-6 is a pleiotropic cytokine, and elevated resting plasma concentrations are associated with CVD and T2DM (Carey and Febbraio, 2004). At rest, the main sources of IL-6 in the circulation are adipose tissue, monocytes, macrophages residing in adipose tissue and the liver (Fantuzzi, 2005). Especially visceral adipose tissue and the residing macrophages within it are a major source of circulating IL-6 at rest (Bastard et al., 2006). Indeed, individuals with obesity have an elevated plasma IL-6 concentration at rest and weight loss can reduce this (Cottam et al., 2004; Maachi et al., 2004). Confirming the association between obesity, inflammation and chronic disease, many observational studies report a positive association between the chronic elevation of plasma IL-6 concentration and insulin resistance, atherosclerosis, T2DM and CVD (Pradhan et al., 2001; Bastard et al., 2006; Dandona et al., 2004; Duncan, 2003; Vozarova et al., 2001). However, IL-6 has also been shown to exert positive effects on inflammation and metabolic health (Pedersen and Febbraio, 2008). Therefore, possibly due to its pleiotropic nature, the exact mechanism underlying the observed
associations between IL-6 and chronic disease are yet incompletely understood (Del Giudice and Gangestad, 2018).

Indicating the diverse actions of IL-6, infusion of IL-6 for 2 to 4 h does not alter (Steensberg et al., 2003) or even enhances insulin sensitivity (Carey et al., 2006), while many cross-sectional studies have reported a relationship between elevated IL-6 concentrations and insulin resistance (Dandona et al., 2004). Possible explanations for this discrepancy are that while short-term elevations of plasma IL-6 concentrations can acutely enhance insulin action via AMP-activated protein kinase activation and GLUT4 translocation (Carey et al., 2006), chronic elevations may impair insulin signalling via the phosphorylation of IRS-1 (Dandona et al., 2004). In this light, it is noteworthy that IL-6 production is upregulated in response to increased concentrations of TNF-α (Stenvinkel et al., 2005). Since the detrimental role of TNF-α in insulin sensitivity and vascular function is relatively well-established (Dandona et al., 2004; Stenvinkel et al., 2005), it is suggested that IL-6 may serve as a bystander rather than having a direct impact on health (Febbraio and Pedersen, 2005). On the other hand, IL-6 promotes the production of CRP, which inhibits the expression of vasodilatory agents (i.e. endothelial nitric oxide synthase) and is a strong independent risk factor for CVD (Ridker, 2003); suggesting a more causative role of IL-6 in the aforementioned negative health outcomes. Moreover, pharmacologically blocking of the IL-6 receptor can alleviate symptoms of a range of inflammatory diseases, such as rheumatoid and juvenile idiopathic arthritis (Kang et al., 2015), but is at the same time associated with increased concentrations of cholesterol and worsening of insulin sensitivity (Febbraio and Pedersen, 2008). The exact function of IL-6 in the circulation may also depend on the receptors it binds following appearance in the circulation. Several cell types (e.g. leukocytes, hepatocytes) express surface IL-6 receptors (IL-6R) for classic IL-6 signalling, while IL-6 can also bind IL-6Rs present in the circulation, after which the resulting complex of IL-6/IL-6R/gp130 can bind tissue that does not express the IL-6R (e.g. skeletal muscle) (i.e. trans-signalling). Mainly the latter signalling pathway is suggested to be associated with inflammation (Del Giudice and Gangestad, 2018). Taken together, although the exact underlying mechanisms need further research,
chronically elevated plasma IL-6 concentrations are associated with poor cardiometabolic health (Bastard et al., 2006; Dandona et al., 2004).

Monocytes

Monocytes are part of the innate immune system and constitute around 5-15% of the total leukocyte count in the circulation. They can phagocytose micro-organisms, present antigens to lymphocytes, but also produce pro-inflammatory cytokines (Akira et al., 2006). The production of these cytokines can be induced via their surface receptors, TLR2, TLR4 and cluster of differentiation (CD)14 (Asea et al., 2000). By binding the TLR-CD14 complex, several pathogen-associated molecular patterns (PAMPs) can trigger the production of cytokines such as IL-6 and TNF-α via the activation of the nuclear factor kappa B (NF-κB) pathway (Asea et al., 2000). As a result, the expression of TLRs on the surface of monocytes has been suggested as a marker for chronic-low grade inflammation (Lancaster et al., 2005). Indeed, monocytes of physically inactive people and individuals with diabetes express more TLR2 and TLR4 when compared with healthy controls (Dasu et al., 2010; Lancaster et al., 2005).

In addition to the surface expression of TLRs, the inflammatory nature of monocytes can be characterised by its expression of CD14 and CD16. Using these 2 surface markers, monocytes can be divided into 3 subtypes: classical monocytes (CD14++CD16-), intermediate monocytes (CD14++CD16+) and non-classical monocytes (CD14+CD16++) (Ziegler-Heitbrock et al., 2010). A flow cytometry panel illustrating the gating strategy to identify each of the 3 subsets is given in Fig. 1.1. A large proportion of intermediate and non-classical monocytes are associated with CVD, T2DM and other chronic diseases (de Matos et al., 2016; Wong et al., 2012), suggesting that the distribution of monocyte subsets can be used as a marker for chronic low-grade inflammation. Indeed, in response to an in-vitro stimulant (i.e. lipopolysaccharide (LPS)) non-classical and intermediate monocytes produce more of the pro-inflammatory cytokines TNF-α and IL-1β than the classical subset (Belge et al., 2002; Mukherjee et al., 2015). In evaluating studies on the inflammatory characteristics of monocyte subsets it should be noted that although the distinction between CD16 positive and CD16
negative monocytes is known for some decades now (Passlick et al., 1989), it is only recently that the third monocyte subset is widely acknowledged (Ziegler-Heitbrock et al., 2010). Therefore, more research is needed to establish the role of this new subtype and the distribution of the 3 monocyte subtypes in chronic low-grade inflammation and chronic diseases.

Fig. 1.1 The 3 monocyte subsets identified by their CD14 and CD16 expression, using flow cytometry. Adapted from Zawada et al., 2015.

**Heat shock protein 72**

Heat shock protein (HSP) was first detected in the salivary glands of Drosophila that had been incubated at elevated temperatures (Rossita, 1962). Since this initial paper, the thousands of papers that followed have reported the presence of HSPs in every eukaryotic cell type and have established the classification of this protein based on their molecular mass, ranging from the HSP10 to the HSP110 family (Noble et al., 2008). With regards to chronic low-grade inflammation and exercise, the HSP70 family, with its inducible subtype Hsp72, is most widely studied and is the HSP of focus in this thesis. Besides its presence in all cells in the human body, Hsp72 is also released into the
Intracellular Hsp72 (iHsp72) functions as a chaperone for protein folding and aids in the maintenance of homeostasis within cells (Noble et al. 2008). When in homeostasis, Hsp72 is bound to heat shock factor-1 (HSF-1) in the cytosol, rendering this complex inactive. In response to physiological stress or inflammation, these molecules are uncoupled, allowing HSF-1 to translocate to the nucleus and activate heat shock elements (HSEs) on the heat shock protein gene. As a result, the transcribed Hsp72 mRNA then leads to an increased Hsp72 protein expression in the cytosol of the cell (Kiang and Tsokos, 1998). Its expression and association with metabolic health has been assessed in a variety of cell types, with levels in leukocytes, adipose tissue and skeletal muscle tissue having gained most attention in the context of chronic low-grade inflammation and metabolic health (Henstridge et al., 2014b). As is summarised in Fig. 1.2, it is suggested that iHsp72 exerts its anti-inflammatory actions by blocking the activity of the C-Jun N-Terminal Kinase (JNK) and NF-κB pathways, reducing the production of pro-inflammatory cytokines and facilitating insulin sensitivity (Chung et al., 2008). Indeed, people with T2DM and non-alcoholic fatty liver disease have lower expression of iHsp72 in skeletal muscle and adipose tissue compared to healthy controls (Bruce et al., 2003; Di Naso et al., 2015; Henstridge et al., 2010) and Hsp72 knock-out mice develop insulin resistance (Henstridge et al., 2014b). At the same time, mice in which iHsp72 is overexpressed are protected against the deleterious effects of high fat overfeeding on insulin sensitivity (Henstridge et al., 2014a) and pharmacologically restoring Hsp72 expression induces an 85% increase in glucose clearance rate during intravenous glucose infusion in monkeys (Kavanagh et al., 2011).
Fig. 2.2 The impact of chronic low-grade inflammation on insulin sensitivity and the suggested effect of elevated intracellular heat shock protein 72 expression. A chronic positive energy balance, eventually resulting in obesity, leads to the activation of the NF-κB and JNK pathways. This results in an enhanced production of pro-inflammatory proteins and inhibits insulin receptor substrates, attenuating insulin sensitivity. Elevated expression of iHsp72 in insulin sensitive tissue, however, can dampen the activation of these pathways, enhancing insulin sensitivity and reducing inflammation. Small arrows = promotes production or activates; bold arrows = produces; bold lines = blocks or attenuates. Abbreviations: NF-κB = nuclear factor kappa B; JNK = c-Jun N-terminal kinase; iHsp72 = intracellular heat shock protein 72; IRS-1 = insulin receptor substrate-1; TNF-α = tumour necrosis factor-α; IL-6 = interleukin-6; IL-1β = interleukin-1β; eHsp72 = extracellular heat shock protein 72.

Although animal studies have provided compelling data on the influence of iHsp72 in skeletal muscle on metabolic health (Chung et al., 2008; Kavanagh et al., 2011), the protective effect of an elevated expression of iHsp72 in circulating immune cells is less clear. Of the leukocyte subtypes, iHsp72 in monocytes is most responsive to stress (Bachelet et al., 1998) and since monocytes produce a range of pro-inflammatory cytokines when activated, iHsp72 levels in this cell type may have important implications for the inflammatory profile of an individual. In addition, like skeletal muscle, monocytes are insulin sensitive and may therefore have potential as a surrogate measure for peripheral insulin sensitivity (Simar et al., 2012a). Simar et al. (2004), Singh et al. (2006) and Njemini et al. (2002) found a reduction in resting iHsp72 expression in monocytes as a result of ageing, a process associated with the development of chronic low-grade inflammation (Baylis et al., 2013).
Furthermore, increased basal expression of iHsp72 in monocyte derived macrophages reduces the production of TNF-α and IL-1β in response to in-vitro LPS stimulation (Ding et al., 2001), supporting the anti-inflammatory function of iHsp72.

Besides basal expression, the inducibility of iHsp72 expression in response to stress may serve as a marker for cardiometabolic health. For instance, the acute iHsp72 increase in monocytes following in-vitro heat shock is impaired in elderly individuals compared with younger adults (Njemini et al., 2002; Singh et al., 2006) and is associated with impaired insulin sensitivity (Chichester et al., 2015). Moreover, although trained runners have lower resting iHsp72 expression (Fehrenbach et al., 2000; Selkirk et al., 2009), these individuals show a larger increase in iHsp72 expression compared with their sedentary counterparts in response to exercise (Selkirk et al., 2009). Together, both basal expression as well as the acute inducibility of iHsp72 expression in monocytes may have potential as a marker for chronic low-grade inflammation (Hooper et al., 2014). However, whether changes in these markers indeed impact on cardiometabolic health needs further investigation.

In contrast to the anti-inflammatory actions of Hsp72 within the cell, Hsp72 in the circulation (eHsp72) can activate monocytes via the TLR4/CD14 complex, inducing the production of pro-inflammatory cytokines (Asea et al., 2000). Indeed, elevated basal levels of eHsp72 are linked to impaired insulin sensitivity (Chichester et al., 2015; Dasu et al., 2010; Krause et al., 2014) and the development of atherosclerosis in individuals with hypertension (Pockley et al., 2003). In further support of its potential role in chronic low-grade inflammation, resting eHsp72 concentrations are strongly correlated with resting serum TNF-α and CRP concentrations in elderly individuals (Njemini et al., 2004). Thus, by stimulating the production of pro-inflammatory cytokines in circulating immune cells, eHsp72 may exacerbate chronic low-grade inflammation and exert a negative effect on health.

Notwithstanding the cross-sectional data to suggest the deleterious role of eHsp72 on several aspects of health, evidence for its potential to induce pro-inflammatory cytokine release in monocytes
and other leukocytes is equivocal (Johnson and Fleshner, 2006). It is suggested that the activation of monocytes following *in-vitro* incubation with eHsp72 is the consequence of contamination with endotoxins rather than the effect of eHsp72 itself (Bausinger et al., 2002; Gao and Tsan, 2003). For instance, incubating monocyte derived dendritic cells with endotoxin-free Hsp70 does not induce an acute inflammatory response (Bausinger et al., 2002). Moreover, pre-incubation of eHsp72 with polymyxin-B to block the actions of the potential contaminant LPS abolishes the production of pro-inflammatory cytokines in macrophages (Gao and Tsan, 2003). Therefore, further *in-vitro* research on the mechanistic actions of eHsp72 should carefully control for possible contamination by endotoxins.

Besides uncertainty about its influence on cytokine production of immune cells, the main tissues that excrete eHsp72 into the circulation are not fully identified (Johnson and Fleshner, 2006). Nevertheless, the liver, the brain and leukocytes are known to release Hsp72 into the circulation via passive as well as active mechanisms (Johnson and Fleshner, 2006). In a study using exercise as a stressor, Febbraio et al. (2002) showed that skeletal muscle does not contribute to circulating eHsp72 concentrations. Therefore, while cross-sectional data implies a detrimental role for chronic elevations of eHsp72 in cardiometabolic health, its mechanistic actions and potential as a marker for chronic low-grade inflammation require further research.

### 1.2 The acute inflammatory response to exercise

The strong evidence for the link between chronic low-grade inflammation and non-communicable diseases (Ridker et al., 2003) makes the first an interesting target to prevent or treat the latter. It is widely acknowledged that regular exercise can be protective against T2DM and CVD (Leung et al., 2008; Sigal et al., 2006). Cross-sectional evidence indeed suggests that regular physical activity can result in lower basal levels of IL-6, eHsp72, intermediate and non-classical monocytes (Gleeson et al., 2011). It has been suggested that the inflammatory and subsequent anti-inflammatory response following each bout of exercise is partly responsible for the protective effects of exercise (Gleeson et al., 2011; Petersen and Pedersen, 2005).
Cytokines

Exercise of sufficient intensity and duration evokes a range of stressors on the body, such as hyperthermia, glycogen depletion, hypoxia and oxidative stress (Pedersen and Febbraio, 2008). As a result of those and other stressors accompanied with exercise, several human tissues respond with the production of cytokines and other proteins, together described by the term “acute inflammatory response” (Gleeson et al., 2011). The main secretors of proteins in response to exercise are the liver, leukocytes and skeletal muscle (Febbraio and Pedersen, 2005). Especially the latter has received much attention in the last ~20 years for its role as an endocrine organ in response to exercise. This has led to the establishment of the term ‘myokines’, which are muscle-derived cytokines (Febbraio and Pedersen, 2005). Of all myokines produced by the muscle, IL-6 responds most dramatically to exercise and has been suggested a main driver of the anti-inflammatory effects of exercise (Petersen and Pedersen, 2005). Plasma IL-6 concentration peaks immediately following the cessation of exercise, which in turn triggers the production of anti-inflammatory cytokines such as IL-1ra, IL-10 (Steensberg et al., 2003), but also iHsp72 (Febbraio et al. 2002), while simultaneously suppressing TNF-a and IL-1β production (Pedersen and Febbraio, 2012). The anti-inflammatory cytokine IL-1ra attenuates the activation of the pro-inflammatory cytokine IL-1β, while IL-10 exerts a range of anti-inflammatory functions, such as stimulating T-regulatory cell production (Gleeson et al., 2011). Moreover, iHsp72 can block the activation of the inflammatory pathways NF-κB and JNK.

Considering their deleterious impact on insulin signalling, the acute and chronic elevation of iHsp72 may play a role in the health promoting effect of exercise (Henstridge et al., 2014). Therefore, the acute inflammatory response, defined as the elevation in plasma IL-6 and eHsp72 concentration immediately after exercise as well as the longer lasting elevation of anti-inflammatory agents such as IL-1ra, IL-10 and iHsp72, may partly explain the health promoting effects of exercise (Gleeson et al., 2011). Of note, the acute inflammatory response following exercise differs from that observed after infection, as generally no increase in the pro-inflammatory cytokines TNF-a and IL-1β is observed following exercise (Petersen and Pedersen, 2005). Throughout this thesis, the ‘acute inflammatory
response’ refers to the acute elevation of cytokines and other proteins in response to the metabolic stress associated with exercise and thermal stress rather than infection or inflammation.

The acute elevation of the plasma IL-6 concentration following exercise is dependent on its intensity and duration (Fischer, 2006). In addition, the observation that running seems to induce a larger response compared to cycling, raises the question whether the larger muscle mass involved in this activity compared with cycling contributes to this finding. However, although the previously held belief that the IL-6 response following exercise was solely the result of muscle damage has been contradicted (Febbraio and Pedersen, 2005), muscle damage might still play a role in the larger inflammatory response to running when compared with exercise largely lacking an eccentric component (e.g. cycling) (Nieman et al., 2014). Nevertheless, cycling at moderate intensity for at least ~30 min is effective in inducing an acute IL-6 response, as well as a subsequent rise in plasma concentrations of anti-inflammatory cytokines such as IL-1ra (Leicht et al., 2016; Paulson et al., 2015). As this response is absent following low-intensity walking (Markovitch et al., 2008), the presence of an exercise intensity threshold for the induction of an inflammatory response is conceivable.

In line with the importance of exercise intensity in the acute inflammatory response to exercise (Nieman et al., 2012), the use of high-intensity interval training (HIIT) has recently regained interest as a time-efficient form of exercise to improve health (Little et al., 2010). In this form of exercise, short efforts lasting between 30 s and 4 min are interspersed with active rest, generally totalling around 20 min of exercise (Little et al., 2010). When matched for power output (PO), HIIT can induce larger elevations in IL-6 than more traditional continuous moderate-intensity exercise (Leggate et al., 2010). Interestingly, even short sessions requiring less than half of the energy expenditure of continuous moderate-intensity can induce a similar IL-6 response (Wadley et al., 2016), making it an appealing, time efficient strategy to reduce chronic low-grade inflammation.

Monocytes

There is limited evidence on the potential of an acute exercise bout to alter the distribution of
monocyte subsets in the circulation. Nevertheless, even 1 min of all-out exercise seems sufficient to alter the distribution of monocyte subsets (Steppich et al., 2000). While most studies report an acute increase in intermediate and non-classical monocytes directly following the cessation of exercise (Hong and Mills, 2008; Steppich et al., 2000; Van Craenenbroeck et al., 2014), elevations in classical monocytes following exercise have been reported as well (Leicht et al. 2016; De Matos et al. 2016).

The exercise intensity may partly explain the differences in these findings, as catecholamines are suggested to drive the recruitment of CD16+ monocytes from the marginal pool into the circulation (Selkirk et al., 2009; Steppich et al., 2000). Indeed, moderate intensity exercise does not induce an acute increase in the proportion of CD16+ monocytes (de Matos et al., 2016; Leicht et al., 2016b), in contrast to strenuous, shorter duration exercise (Hong and Mills, 2008; Steppich et al., 2000; Van Craenenbroeck et al., 2014). Whether the relative reduction in these same monocyte subsets reported at 2 h following exercise (Leicht et al., 2016b) is the result of their recruitment to sites of local exercise-induced inflammation is not clear. In addition, it should be noted that the studies of Steppich et al. (2000), Hong et al. (2008) and De Matos et al. (2016) were conducted before the change in nomenclature of the monocyte subtypes (Heitzbroek et al. 2010) and therefore did not include the intermediate monocyte subset in their analysis.

**Heat shock protein 72**

The heat shock response (i.e. the induction of heat shock protein in response to a stressor) is a highly conserved response in animals and is important for survival following acute stress (Li et al. 1983). Exercise is accompanied by a range of stressors that can increase the production of iHsp72, notably hyperthermia, oxidative stress and glycogen depletion (Noble et al., 2008). In turn, iHsp72 plays a pivotal role in the acute response to these physiological stressors by aiding in the refolding of denatured protein and exerting its anti-inflammatory actions e.g. by reducing NF-κB and JNK activation (Hooper et al., 2014; Noble et al., 2008). Therefore, the acute increase in iHsp72 expression following a bout of exercise may be part of the anti-inflammatory effect of exercise that is suggested to contribute to its protective effect against chronic low-grade inflammation (Gleeson et al., 2011; Petersen and Pedersen, 2005).
Several methods to assess changes in iHsp72 expression in circulating immune cells following exercise exists, with Western blotting and flow cytometry most commonly used (Yamada et al., 2008). Using the latter technique, Fehrenbach et al. (2000) reported significant increases in iHsp72 in monocytes and granulocytes of trained runners immediately following a half-marathon. Studies using similar duration exercise have corroborated the finding that acute exercise can indeed increase iHsp72 expression in circulating immune cells (Hillman et al., 2011; Peart et al., 2013). Whether there exists a minimum volume of exercise needed to induce increases in iHsp72 expression is currently unclear. Shastry et al. (2002) and Lee et al. (2014) reported no increase in iHsp72 expression following a shorter (1 h; Shastry et al., 2002) or lower intensity exercise bout (50% VO2peak; Lee et al., 2014) compared with the aforementioned studies. On the other hand, 4 min of all-out cycling induces a significant increase in iHsp72 expression (Peart et al., 2011). The latter finding may be explained by the oxidative stress and metabolic disturbance caused by all-out exercise. Indeed, the increase in iHsp72 occurred together with an increase in plasma concentrations of a marker for oxidative stress (i.e. thiobarbituric acid substances) (Peart et al., 2011). Interestingly, supplementation with sodium bicarbonate blunts the oxidative stress response as well as the increase in iHsp72 (Peart et al., 2011). Collectively, longer duration or more intense exercise seems needed to induce an acute iHsp72 response when compared with IL-6 and a change in the monocyte subset distribution.

Besides the elevation of iHsp72 levels, Hsp72 is also secreted into the circulation in response to exercise-induced stress. The exact trigger for an increase in eHsp72 release into the circulation following exercise remains unclear (Johnson and Fleshner, 2006). While this was initially thought of to result from cell necrosis following exercise-induced cell damage, it is now acknowledged that eHsp72 can also be released via active mechanisms (Whitham, 2008). In support, downhill running, which can lead to muscle damage due to its large eccentric component, does not amplify the acute eHsp72 response compared with running on the flat (Peake et al., 2005). Moreover, concentric endurance exercise, resulting in limited muscle damage, can induce increases in eHsp72 concentrations (Whitham, 2008). Suggested mechanisms of active release in response to exercise are via lipid rafts or exosomes (Murshid & Calderwood, 2012). Indeed, the role of exosomes in tissue and
organ cross-talk is increasingly recognised (Whitham et al., 2018), providing a potential mechanism in which eHsp72 exerts its immuno-stimulatory effects after exercise (Johnson and Fleshner, 2006).

As with iHsp72, the release of eHsp72 after exercise seems dose-dependent. For example, by directly comparing a range of running bouts of different durations and intensities, Fehrenbach et al. (2004) showed a markedly larger increase in eHsp72 after a marathon compared to shorter duration continuous and interval runs. Nevertheless, it should be noted that all sessions investigated in this study were challenging in nature and not suitable for less trained or motivated individuals. Similarly, other studies reporting a significant increase in eHsp72 following exercise have used exercise of 60 min or more (Walsh et al., 2001; Whitham et al., 2007), leaving the question whether smaller volume exercise can induce increases in eHsp72 concentrations.

Since eHsp72 can induce cytokine production by monocytes (Asea et al., 2000), it is suggested that the acute increase in eHsp72 concentrations contributes to the IL-6 response following exercise (Fehrenbach et al., 2005). In neutrophils, incubation with Hsp72 concentrations reported following exercise results in an enhanced phagocytic capacity (Giraldo et al., 2010). In contrast, Starkie et al. (2001) found no increased expression of intracellular IL-6 in monocytes following prolonged cycling, leading them to conclude that monocytes are not the source of circulating IL-6 following exercise. Although skeletal muscle seems the main source of circulating IL-6 following exercise (Steensberg et al., 2000), eHsp72 may modulate part of the acute inflammatory response following exercise by stimulating circulating immune cells (Asea et al., 2000).

### 1.3 Chronic exercise training to reduce chronic low-grade inflammation

Exercise can induce an anti-inflammatory milieu in the hours after the session. Following the theory postulated in several reviews (Gleeson et al., 2011; Petersen and Pedersen, 2005), this acute response may eventually reduce chronic low-grade inflammation when engaging in exercise on a regular basis. To date, cross-sectional and longitudinal studies lend support for this theory (Beavers et al., 2010; Hamer et al., 2012; Wilund, 2007). However, exercise training results in a range of adaptations, such as weight loss and an increased muscle mass, and it is not yet clear to what extent
these adaptations mediate the reductions in low-grade inflammation seen after chronic interventions. Moreover, the exact mechanisms underlying changes in the resting inflammatory profile need further study. Possible candidates to explain improvements in the inflammatory profile after exercise training are reductions in visceral adipose tissue (producing pro-inflammatory cytokines at rest) (Fantuzzi, 2005), reduced TLR expression on immune cells (Flynn and McFarlin, 2006) and changes in the number and phenotype of circulating cells and those residing in tissue (Timmerman et al., 2008) (Fig 1.3). This thesis focusses on circulating IL-6, the monocyte subset distribution, iHsp72 in monocytes and eHsp72 concentration as markers of chronic low-grade inflammation.

Fig. 1.3 Possible explanations for the anti-inflammatory effects of exercise training. Adapted from Gleeson et al., 2011. Abbreviations; IL = interleukin, TNF = tumour necrosis factor, CD = cluster of differentiation, TLR = Toll-like receptor, Treg = T regulatory.
Cytokines

Self-reported physical activity levels are negatively related to basal IL-6 concentration (Reuben et al., 2003; Taaffe et al., 2000). In a longitudinal study, Hamer et al. (2012) showed that physically active individuals had lower resting IL-6 concentrations over a 10-year follow-up period compared to their more sedentary counterparts. The association was still present after adjustment for BMI and waist-to-hip ratio, supporting the anti-inflammatory effect of exercise regardless of changes in body composition. Evidence from intervention studies on the effectiveness of exercise training to lower basal IL-6 levels is less clear (Beavers et al., 2010). Although exercise training can reduce resting IL-6 concentrations, this may be mediated by the weight loss accompanying those exercise programs (Beavers et al., 2010). For instance, a low-energy diet is more effective in reducing low-grade inflammation than exercise in patients with coronary artery disease (Pedersen et al., 2016). In addition, the duration of exercise interventions may influence their effectiveness in reducing resting IL-6 concentrations. While studies with a duration shorter than 10 months show mixed results, those of 24 months consistently report reductions in resting IL-6 concentrations (Beavers et al., 2010). Finally, the inflammatory profile at baseline can influence the changes induced by exercise training, as for instance CRP concentrations after a chronic exercise intervention are only reduced in those starting with elevated plasma concentrations (Lakka et al., 2005).

Monocytes

Cross-sectional data suggest that monocytes in the circulation of physically active individuals consist of a smaller proportion of pro-inflammatory monocytes (i.e. non-classical and intermediate monocytes) compared to their more sedentary counterparts (Timmerman et al., 2008). Limited exercise intervention studies exist with monocyte subtypes as an outcome measure. Nonetheless, 2 weeks of HIIT do not change the distribution of monocyte subsets in overweight and obese males (Oliveira-Child et al., 2013). Conversely, 12 weeks of mixed endurance- and resistance-type training decreases the proportion of the pro-inflammatory monocyte subsets in previously sedentary individuals (Timmerman et al., 2008). As the latter study was performed prior to the consensus statement on the identification of a third monocyte subset (Ziegler-Heitbrock et al., 2010), more long-
term exercise intervention studies are needed to examine the effect of exercise on the distribution of the 3 monocyte subsets.

Heat shock protein 72

While exercise training can increase iHsp72 expression in skeletal muscle (Morton et al., 2009), trained runners have a lower basal iHsp72 expression in circulating immune cells compared with sedentary individuals (Fehrenbach et al., 2000; Selkirk et al., 2009). The latter is suggested to be an adaptation to the reduced oxidative stress present in the trained runners, resulting from regular exercise training (Fehrenbach et al., 2000). In support of this notion, iHsp72 expression in monocytes is lower in physically active compared to physically inactive elderly (Simar et al., 2007). Therefore, exercise training might exert different effects on resting iHsp72 expression in monocytes compared to skeletal muscle. Unfortunately, there is a lack of chronic exercise intervention studies on iHsp72 in monocytes to underpin this suggestion (Yamada et al., 2008). Nevertheless, an 8-week walking programme in elderly individuals reduces iHsp72 expression in monocytes (Simar et al., 2012b).

Despite the growing evidence for acute changes in eHsp72 concentrations following exercise (Fehrenbach et al., 2005; Walsh et al., 2001), there are limited data on the potential of exercise training to reduce eHsp72 concentrations. As eHsp72 can activate circulating monocytes, reductions in resting pro-inflammatory cytokines may be mediated by reductions in eHsp72 concentrations. While conditions that are generally associated with low physical activity levels (e.g. insulin resistance) are associated with elevated resting eHsp72 concentrations (Krause et al., 2014), no direct study has investigated the relationship between physical activity levels and this marker. However, 2 resistance training interventions in elderly individuals showed a reduction in resting eHsp72 concentrations alongside more traditional inflammatory markers such as IL-6 (Ogawa et al., 2010; Perreault et al., 2016). Therefore, the limited existing evidence suggests that exercise training can reduce the eHsp72 concentration. It should be noted, however, that the reported increases in plasma concentrations were small and could have been the result of an increase in plasma volume following the intervention.
1.4 Chronic low-grade inflammation in wheelchair users

While the number of people suffering from T2DM and CVD in the general population has become increasingly alarming (Wild et al., 2004), the prevalence of these diseases in wheelchair users, notably individuals with SCI, is even higher (Bauman and Spungen, 2008). Furthermore, resting concentrations of pro-inflammatory cytokines are also elevated in this population (Frost et al., 2005; Manns et al., 2005; Wang et al., 2007). Although the latter might be the result of acute infection, catheter use or medication in a population suffering from many comorbidities as a result of the injury (Frost et al., 2005), Wang et al. (2007) shows that findings of an unfavourable inflammatory profile in people with SCI still holds true when controlling for these factors. In addition, in a large cohort study, people with SCI matched for age and medication use, showed lower levels of naïve T-cells than able-bodied subjects (Pavlicek et al., 2017), suggesting that SCI indeed impacts on a range of factors related to immune function.

A lesion of the spinal cord, and thus the interruption of the descending nerves from the brain, leads to paralysis of skeletal muscle below the lesion, the loss of afferent sensory feedback and the inhibition of sympathetic innervation of the tissue below the lesion (Krassioukov, 2009). A spinal lesion can be classified as a cervical spinal cord injury (CSCI) or paraplegia (a spinal lesion below the cervical level). A CSCI impacts on sensory and motor control of all limbs, while a paraplegia mainly affects the lower limbs and in some cases the trunk (i.e. when lesion is above lumbar (L)1) (Martini and Nath, 2011). Sympathetic innervation of the nerves below the lesion is affected in both people with CSCI and a paraplegia above the level of L1, disrupting the innervation of blood vessels and sweat glands (Krassioukov, 2009). In people with a lesion above the level of thoracic 6 (T6), sympathetic innervation of the heart and the adrenal medulla is also affected. The lack of sympathetic innervation of the heart and blood pooling in the legs resulting from impaired blood vessel innervation results in a reduced capacity to match cardiac output with the demands of exercise, potentially leading to reduced exercise capacity in people with CSCI (West et al., 2013). In addition, sympathetic tone and catecholamine concentrations at rest are lower in people with a complete lesion above the level of T6 (Krassioukov, 2009). Therefore, the deterioration of physical capacity and the comorbidities
associated with a SCI are dependent on the lesion level, with a higher lesion exerting the most severe impact (Haisma et al., 2006). Appreciating the comorbidities and changes in lifestyle associated with SCI, it is difficult to make firm conclusions on the effect of a spinal lesion per se on alterations in immune function and chronic low-grade inflammation. However, considering the anti-inflammatory functions of catecholamines (e.g. adrenaline reduces the acute inflammatory response to LPS and stimulates IL-10 production)(Elenkov et al., 2000), it is conceivable that the elevated risk for chronic low-grade inflammation in this population is at least partly related to sympathetic dysfunction.

The paralysis of skeletal muscle below the lesion and the additional consequences of a SCI result in a lower physical capacity and the dependency on the upper-body for daily activities, exercise and transport. Together with the environmental, social and physical barriers that are imposed by the dependency on a wheelchair for locomotion (Vissers et al., 2008), this can make engaging in sufficient exercise or physical activity problematic. Indeed, individuals with SCI are more inactive when compared with the general population (Martin Ginis et al., 2010). In addition, paralysis of the tissue below the lesion and being wheelchair bound reduced basal metabolic rate and daily energy expenditure, making it more difficult to maintain energy balance (Monroe et al., 1998). Therefore, the elevated basal levels of pro-inflammatory cytokines reported in people with SCI (Wang et al., 2007) may be a consequence of the injury itself, but could also be mediated by the lower levels of physical activity and changes in body composition generally seen in those with SCI (Maggioni et al., 2003; Martin Ginis et al., 2010; Morse et al., 2008).

1.5 Exercise modalities for wheelchair users

Due to paralysis below the lesion, most people with SCI are reliant on their upper body for exercise. Although active modes of transport using the upper body exist for several centuries, participation in sport and physical activity of wheelchair users has become increasingly popular since the Stoke Mandeville Games in 1948 (Webborn and Van De Vliet, 2012). This has led to the creation of several wheelchair sports and modes of physical activity suitable for the upper body. Wheelchair sports can be categorised in court sports such as wheelchair rugby, tennis and basketball, and
endurance sports such as handcycling and wheelchair racing. In addition, ergometers suitable for wheelchair users have made exercise in rehabilitation settings increasingly accessible (Valent et al., 2008). A widely used ergometer is the arm-crank ergometer, which mimics the asynchronous movement pattern known from the cycle ergometer, performed by the arms instead of the legs.

As upper-body exercise engages a significantly smaller muscle mass compared to whole- or lower-body exercise, the physiological strain during this type of activity differs from lower-body exercise such as cycling. At the same absolute intensity (i.e. same PO), upper-body exercise results in a higher oxygen uptake (\(\dot{V}O_2\)), HR and rating of perceived exertion (RPE) when compared with lower-body exercise. At the same relative intensity (i.e. the same percentage of peak PO (PO\(_{\text{peak}}\)) or \(\dot{V}O_2\) (\(\dot{V}O_2\)\(_{\text{peak}}\))), however, these parameters are lower during upper-body exercise. At maximal exertion \(\dot{V}O_2\) during arm-cranking equates to ~60% of that reported during cycling (Sawka, 1986). Blood lactate concentrations, on the other hand, are similar or higher during upper-body exercise at the same relative intensity when compared with lower-body exercise, possibly resulting from a larger proportion of type II muscle fibres and a smaller diffusion area in skeletal muscle of the arms (Sawka, 1986). The discrepancy between central and peripheral physiological strain during upper-body exercise is also reflected in the differentiated RPE during exercise (i.e. RPE reported for both central and local fatigue). During tests to volitional exhaustion, local RPE exceeds central RPE during the incremental stages of the test and is therefore suggested to dominate in the perception of effort during upper-body exercise (Lenton et al., 2008). It should be noted, however, that the difference between local and central physiological strain might depend on the training status of the individual (Jacobs et al., 2013). Since members of the general population as investigated in Lenton et al. (2008) are not accustomed to upper-body endurance exercise, caution needs to be exercised when translating findings from studies on able-bodied individuals to those chronically trained in the upper body, such as wheelchair users.

Despite the smaller central physiological strain during upper-body exercise when compared with lower-body exercise, arm-crank ergometry training programmes increase \(\dot{V}O_2\)\(_{\text{peak}}\) and PO\(_{\text{peak}}\) in wheelchair users (Van der Scheer et al., 2017). In addition, improvements in body composition and
traditional biochemical health markers such as circulating triglycerides and low-density lipoproteins are reported (van der Scheer et al., 2017). Promisingly, this is even the case in people with CSCI early during rehabilitation (Valent et al., 2008). As a result, an increasing number of institutes promote exercise and physical activity in wheelchair users (Martin Ginis et al., 2018), both during the rehabilitation phase (Nooijen et al., 2012) as well as thereafter (Hoekstra et al., 2017). Notwithstanding the relatively well-established effectiveness of upper-body endurance exercise to improve physical fitness in wheelchair users (Van der Scheer et al., 2017), less is known about the potential of upper-body exercise to reduce chronic low-grade inflammation.

1.6 The acute inflammatory response to upper-body exercise

Studies using lower-body exercise suggest that the acute inflammatory response is intensity and duration dependent (Fischer, 2006). As exercise intensity indirectly reflects the involved muscle mass, it is suggested that the latter also impacts on this acute response (Fischer, 2006). Therefore, the relatively small muscle mass involved in upper-body exercise might attenuate the acute inflammatory response and, as a result, its potential to combat chronic low-grade inflammation. Indeed, early research pointed towards a limited inflammatory response following arm-exercise (Bergfors et al., 2005; Hirose et al., 2004; Nosaka, 1995). However, later studies using protocols of longer duration and higher intensities showed that also upper-body exercise can induce an increase in IL-6 and the subsequent rise in anti-inflammatory cytokines (Kouda et al., 2012; Paulson et al., 2014; Umemoto et al., 2011). In able-bodied individuals, Kouda et al. (2012) showed that 20 min of arm-cranking at 60% \( \dot{V}O_{2\text{peak}} \) induced an acute ~1.7 fold elevation in IL-6 concentrations, while 2 h of arm-cranking at the same relative intensity resulted in an almost 10-fold increase (Umemoto et al., 2011). In the latter study, a similar IL-6 response was found in participants with paraplegia, suggesting that also in wheelchair users exercise can induce an acute inflammatory response. This notion has since been replicated in a variety of settings, including a wheelchair-marathon (Ogawa et al., 2014; Sasaki et al., 2014).
Although upper-body exercise of sufficient volume can induce an acute inflammatory response, it is less clear how this response compares to that observed following lower-body exercise. Nevertheless, Helge et al. (2011) showed a higher IL-6 release from the arms compared to the legs during whole-body exercise, suggesting that active muscle mass per se is not a determining factor in the acute inflammatory response to exercise. Indeed, Paulson et al. (2015) found no additional increase in the acute IL-6 and IL-1ra response when low-intensity cycling was added to arm-cranking compared with arm-cranking only. Moreover, in a direct comparison between arm-cranking and cycling for 45 min at 60% $\dot{V}O_{2}\text{peak}$, Leicht et al. (2016) reported similar IL-6, IL-1ra and monocyte subset responses following both modalities. Together, this suggests that when performed at the same relative intensity, upper-body exercise induces an acute inflammatory response of similar magnitude as lower-body exercise. However, it should be noted that the aforementioned studies have all included able-bodied participants unaccustomed to upper-body exercise. As a result, this form of exercise, even when performed at the same relative intensity, might result in a larger stress response in the exercising muscle than a more familiar exercise modality such as cycling. For instance, untrained individuals show an exacerbated oxidative stress response following a bout of running compared to trained runners (Falone et al., 2010). Since even sedentary wheelchair users are more adapted to upper-body exercise than able-bodied individuals (Jacobs et al., 2013), the role of local training status (i.e. adaptations in the involved skeletal musculature) in the acute inflammatory response to upper-body exercise deserves further attention.

The acute inflammatory response to exercise in people with CSCI

Although upper-body exercise induces an acute inflammatory response in AB individuals and those with paraplegia, this response is attenuated in individuals with CSCI (Kouda et al., 2012; Paulson et al., 2013). The attenuated inflammatory response in this group might be due to the smaller active muscle mass and a dampened catecholamine response to exercise (Kouda et al., 2012; Paulson et al., 2013). A lesion above the level of T6 results in an impaired sympathetic innervation of the heart and adrenal medulla (Claydon and Krassioukov, 2008). The latter results in an attenuated adrenaline and noradrenaline production in response to exercise (Claydon and Krassioukov, 2008).
Observational studies in able-bodied individuals have reported a correlation between IL-6 and adrenaline concentrations following exercise (Nieman et al., 2012; Papanicolaou et al., 1996). On the other hand, infusion of adrenaline at similar concentrations as reported during exercise results in a smaller IL-6 response compared with exercise (Steensberg et al., 2001), suggesting that additional stressors are involved in exercise that induce IL-6 release into the circulation. Nevertheless, in people with CSCI, the attenuated adrenaline response in CSCI reported by Paulson et al. (2013) and Kouda et al. (2012) was accompanied with a markedly dampened IL-6 response. While an attenuated catecholamine response to exercise is reflective of autonomic dysfunction, additional measures of autonomic function are available. In addition, the occurrence of spinal reflexes during exercise can result in spill-over of noradrenaline into the circulation, even in people with a complete CSCI (West et al., 2016). Therefore, for a more complete understanding of the influence of autonomic dysfunction in people with CSCI on the acute inflammatory response additional measures independent of exercise stress are needed. Examples of such tests are the sit-up tilt test and the sympathetic skin response test. The first test provides insight in the autonomic modulation of the heart and blood pressure at rest and in response to a sit-up tilt manoeuvre, while the latter assess the remaining sympathetic innervation of the skin. Using the latter test, West et al. (2013) showed that autonomic dysfunction is associated with impaired endurance as well as sprint performance in wheelchair athletes (West et al., 2013). Whether such a test is predictive of the acute inflammatory response to exercise is currently unknown. Taken together, more research is needed to depict the underlying reasons for the dampened acute inflammatory response to exercise in people with CSCI.

### 1.7 Chronic upper-body exercise to reduce chronic low-grade inflammation

Cross-sectional studies indicate that regular physical activity improves the resting inflammatory profile (Morse et al., 2008) as well as metabolic markers such as fasting glucose and insulin in people with SCI (Buchholz et al., 2009). Interestingly, the protective effects of physical activity against chronic-low grade inflammation have been found to be more pronounced in people with paraplegia compared to those with CSCI (Buchholz et al., 2009). In contrast, evidence for no relationship between fitness or physical activity levels and resting concentrations of pro-inflammatory
cytokines in people with SCI exists (Manns et al., 2005). Although this latter study had a relatively small sample size, it suggests that physical activity *per se* does not always lead to an improved inflammatory profile in this population.

Aiming to provide more insight in the potential of exercise to reduce chronic low-grade inflammation in people with SCI, several intervention studies in this population have been conducted (Bakkum et al., 2015; Rosety-Rodriguez et al., 2014; Totosy de Zepetnek et al., 2015). A 12-week arm-crank exercise programme consisting of 3 sessions per week lowers resting concentrations of TNF-α and IL-6 in people with paraplegia (Rosety-Rodriguez et al., 2014), while both hybrid cycling and handcycling reduces resting concentrations of CRP and IL-6 following a 16-week training programme (Bakkum et al., 2015). In the latter study, improvements were reported for both participants with paraplegia as well as CSCI. It is noteworthy that in both studies fat mass was reduced, which could have mediated the lowering in resting concentrations of the inflammatory markers (Gleeson et al., 2011). For instance, body composition was not improved following a study in which the exercise guidelines for people with SCI (Ginis et al., 2011) were followed for 16 weeks (Totosy de Zepetnek et al., 2015). Here, no significant reductions in inflammatory markers at rest were observed. Therefore, although intervention studies show promise for the use of exercise to reduce chronic low-grade inflammation in people with SCI, more research is needed to study the anti-inflammatory potential of upper-body exercise independently of changes in body composition.

Taken together, upper-body exercise can induce an acute inflammatory response of similar magnitude as lower-body exercise when performed at the same relative intensity. However, autonomic dysfunction or a reduced physical capacity may impair the potential of upper-body exercise to induce this acute response. This may make exercise less effective in reducing chronic low-grade inflammation in a range of populations. Therefore, interventions that could amplify the acute inflammatory response may enhance the health promoting effects of exercise. Further elevating body temperature during exercise may be such a factor.
1.8 The acute inflammatory response to exercise in the heat

During endurance-type exercise, the majority of metabolic work produced is converted into heat as a result of the inefficiency of metabolic processes needed for muscle contraction (Dill et al., 1931). While exercise of sufficient intensity in thermoneutral conditions results in an initial rise in core temperature ($T_{core}$), the concomitant vasodilation, increase in skin blood flow and increase in sweat rate can maintain body temperature in a steady state following the initial rise in $T_{core}$. However, when heat dissipation cannot compensate for the metabolic heat produced during exercise, $T_{core}$ will continue to rise (Dill et al., 1931). Core temperature is routinely measured at several sites in the body, such as the rectum, the gastrointestinal tract and the tympanic membrane (Lim et al., 2008). While the measurement of tympanic temperature during exercise can be problematic due to environmental influences (Casa et al., 2007), rectal thermometers and telemetry pills are considered accurate and reliable measures of $T_{core}$ (Lim et al., 2008).

The rise in $T_{core}$ during exercise depends on exercise intensity, but also the environment in which it is performed. For instance, cycling at 60% $\dot{V}O_{2peak}$ at 38°C results in a $T_{core}$ increase of ~2.5°C compared to only 0.7°C when the exercise is performed at 8°C (McFarlin and Mitchell, 2003). Although the influence of a hot environment and the subsequent increase in body temperature on exercise performance have been recognised for a long time now (Adolph et al., 1947), research on the impact of the environment on acute immune responses to exercise has emerged only relatively recently (Shephard, 1998). Nevertheless, when focussing on the acute inflammatory response to exercise, it is evident that exercise in the heat results in an exacerbated inflammatory response when compared with exercise in thermoneutral or cold conditions (Laing et al., 2008; Starkie et al., 2005; Rhind et al., 2004).

Cytokines

Several studies have used a thermal-clamp model (using water-based cycling to successfully clamp $T_{core}$) to investigate the effect of $T_{core}$ on the inflammatory response to exercise (Cross et al., 1996; Laing et al., 2007; Rhind et al., 2004). These studies show that the IL-6 response is significantly
larger after exercise in the heat compared with the cold. Moreover, clamping $T_{core}$ can completely abolish the IL-6 response after cycling in cold water (Rhind et al., 2004). The importance of a rise in $T_{core}$ for the IL-6 response is also shown in more applied exercise settings (i.e. on land), with a dampened increase in IL-6 concentrations when the rise in $T_{core}$ is limited by a cool environment (Starkie et al., 2005). Although temperature elevations can independently induce IL-6 production in skeletal muscle (Welc et al., 2012), the amplified acute cytokine response following exercise in the heat may be partly mediated by the increased plasma catecholamine concentrations (Rhind et al., 2004) and carbohydrate utilisation when compared with exercise in thermoneutral or cold conditions (Fink et al., 1975). This is supported by the dampened IL-6 response in people with interrupted sympathetic activation of the adrenal medulla (i.e. individuals with CSCI), and when carbohydrates are consumed before (Bishop et al., 2001) or during exercise (Nieman, 1998), respectively. During upper-body exercise, however, an additional increase in heat storage induced by the use of a non-permeable suit does not lead to an enhanced IL-6 response (Leicht et al., 2017). As $T_{core}$ was not significantly increased by wearing the suit, this may be indicative of the importance of $T_{core}$ per se with regards to the IL-6 response to exercise.

Heat shock protein 72

Using partly the same participants as in the study of Laing et al. (2008), Whitham et al. (2007) showed that also the eHsp72 response to endurance-type exercise is exacerbated in the heat. Noteworthy, however, is that the eHsp72 response following exercise was not completely abolished when $T_{core}$ was clamped, suggesting other stressors related to exercise are involved in the eHsp72 response. In contrast, Gibson et al. (2014) found a significant increase in eHsp72 concentrations following exercise in heat, but not in temperate or cold conditions. In addition, in the latter study peak $T_{core}$ and the rate of increase in peak $T_{core}$ were the strongest predictors for the eHsp72 response following cycling at 50% $\dot{V}O_{2peak}$. The difference between both studies might have been the exercise modality (cycling versus running), the intensity of the exercise (50% $\dot{V}O_{2peak}$ versus 60% $\dot{V}O_{2peak}$) or the method of $T_{core}$ increase (environmental chamber versus immersion in hot water). Only 1 study has investigated the effect of body temperature during upper-body exercise (Leicht et al., 2017). The
larger increase in heat storage, but not Tcore, resulting from wearing a non-permeable suit led to a significant increase in eHsp72 in contrast to no increase following exercise without the suit (Leicht et al., 2017).

An acute exercise bout in the heat can elevate iHsp72 expression in leukocytes (Fehrenbach et al., 2001; Tuttle et al., 2017), peripheral mononuclear cells (PBMCs) (Kuennen et al., 2011), monocytes (Fehrenbach et al., 2001; Lee et al., 2017) and skeletal muscle (Tuttle et al., 2017). For instance, 45 min of exercise on the treadmill at 50% VO2peak in 47℃ results in a significant increase in iHsp72 expression in PMBCs 4 h post-exercise (Kuennen et al., 2011) and exercise until exhaustion in 30℃ leads to a significant increase in iHsp72 in monocytes 1 h post-exercise (Ruell et al., 2014). Furthermore, 60 min of running in 28℃ results in higher iHsp72 expression in leukocytes 24 h after exercise when compared with running in 18℃ (Fehrenbach et al., 2001), while a hot environment (30-32℃ versus 10-12℃) also enhances the iHsp72 mRNA upregulation in lymphocytes after a 45 min run (Mestre-Alfaro et al., 2012). It is noteworthy that although heat amplified the acute iHsp72 response in the aforementioned studies, significant elevations of iHsp72 expression are also observed following exercise in thermoneutral or cold conditions (Fehrenbach et al., 2001). In line with the notion that the exercise-induced iHsp72 response is influenced by more than hyperthermia only, Hillman et al. (2011) reported that cycling in the heat (34℃) does not induce a larger iHsp72 response in monocytes compared with exercise in a thermoneutral environment (23℃). An interesting additional finding of this study was that dehydration does not impact on the iHsp72 response in monocytes after exercise (Hillman et al., 2011). Together, although hyperthermia can amplify the iHsp72 response to exercise, its magnitude seems also dependent on additional exercise-induced stressors (e.g. glycogen depletion, acidosis and oxidative stress).

Besides its proposed role in chronic low-grade inflammation (Henstridge et al., 2014b), the acute iHsp72 response during exercise in the heat has received increased attention for its protective role against exertional heat illness, possibly via maintaining protein integrity in response to stressors such as hyperthermia (Kregel and Sieck, 2002). Indeed, the survival rate in mice subjected to heat
shock is higher when iHsp72 expression is elevated by a prior non-lethal heat shock compared to control mice (King et al., 2002). While the acute elevation of iHsp72 expression thus has a protective function, adaptations to heat stress may reduce the magnitude of this acute response to large elevations in body temperature. For instance, Tuttle et al. (2017) showed a reduced acute iHsp72 response to an exercise bout in leukocytes after preconditioning with a bout of downhill running in the heat. In support, leukocytes with increased iHsp72 expression following in-vitro heat stress show an impaired ability to further elevate iHsp72 expression in response to a stressor (Ryan et al., 1991). Furthermore, a 10-day heat acclimation programme results in an attenuation of the iHsp72 response in total leukocytes following a 90-min heat stress test (Magalhães et al., 2010). Therefore, while long-term training (Selkirk et al., 2009) or improved physical fitness (Njemini et al., 2002) may enhance the acute iHsp72 response to physiological stressors, heat acclimation and preconditioning can dampen this response (Amorim et al., 2015; Lee and Thake, 2017; Magalhães et al., 2010; Sareh et al., 2011). Although speculative, it may be that the elevated baseline levels of iHsp72 following preconditioning or heat acclimation reduce the need for a further increase in iHsp72 expression to maintain homeostasis within the cell (Sareh et al., 2011).

1.9 Chronic adaptations to exercise in the heat

While several studies have investigated the acute effect of exercise in the heat on inflammatory markers (Laing et al., 2007; Whitham et al., 2007), intervention studies involving exercise in the heat focusing on chronic low-grade inflammation are scarce. Nevertheless, heat acclimation studies provide some insight in the effect of exercise training in the heat on inflammatory markers. Heat acclimation has become a commonly used method to prepare athletes and soldiers for competition or missions in the heat (Taylor and Cotter, 2006). Programmes preparing for performance in the heat generally consist of between 5 and 21 days of exercise sessions lasting more than 60 min, with the exercise intensity set in a variety of ways (e.g. fixed HR or fixed Tcore) (Taylor and Cotter, 2006). The increase in plasma volume, sweat rate and skin blood flow as well as a lower resting and exercising Tcore and HR are coined as the main underlying reasons for enhanced performance in the
heat and protection against exertional heat illness following heat acclimation (Périard et al., 2016). Recently, however, its impact on basal iHsp72 expression has received increased attention as a mediator for the enhanced heat tolerance after heat acclimation (Kuennen et al., 2011). While an increase in basal iHsp72 expression may indeed influence adaptations needed to perform in the heat (Kuennen et al., 2011), the link between iHsp72 and health markers such as insulin sensitivity (Henstridge et al., 2014b) suggests that the implications of heat acclimation-type exercise training may be wider.

*Heat shock protein 72*

In a review, Amorim et al. (2015) showed that exertional heat acclimation programmes can indeed elevate resting iHsp72 expression in circulating leukocytes. However, the potential of these interventions to do so may be dependent on their duration (Amorim et al., 2015). While 3 days of heat acclimation do not increase basal iHsp72 expression in PBMCs (Marshall et al., 2007), 10 days of heat acclimation seem sufficient to increase basal iHsp72 expression in PBMCs (Amorim et al., 2011; McClung et al., 2008). Likewise, the accumulation of iHsp72 in monocytes started only after the third day of heat acclimation and was higher after 10 compared to 3 days of heat acclimation (Lee et al., 2017). Interestingly, no plateau was reached after 10 days of heat acclimation, suggesting that longer heat exposure may result in a further increase in iHsp72 expression.

In contrast to the elevation in resting iHsp72 expression, the effect of heat acclimation on resting eHsp72 concentration is less clear. During 2 days of heat acclimation, resting plasma eHsp72 concentrations decrease after both days of cycling in 38°C at 42% \(\dot{VO_2}_{peak}\) (Marshall et al., 2006). However, no changes in eHsp72 after 10 days heat acclimation are reported (Lee et al. 2017). Speculatively, a change in resting eHsp72 may be a sign of early adaptations to heat stress, returning to pre-acclimation concentrations later on in the intervention. Most exertional heat acclimation studies investigating immunological adaptations have focussed on Hsp72. The limited body of evidence on the other markers of focus in the present thesis suggests that the relatively short-duration heat acclimation interventions are insufficient to alter basal plasma IL-6 concentrations (Kuennen et al.,
To date, no data exist on the effect of chronic exertional heat stress on the distribution of monocyte subsets. Taken together, heat acclimation interventions, typically of relatively short duration but involving frequent intense exercise, increase resting iHsp72 expression in circulating leukocytes and may reduce resting eHsp72 concentration. It is not known whether such programmes can have health implications beyond the protection against exertional heat illness. Given the role of iHsp72 in insulin sensitivity and vascular function (Henstridge et al., 2014b), this may be a fruitful area of future research.

1.10 The acute inflammatory response to passive heating

As body temperature partly mediates the acute inflammatory response to exercise (Laing et al., 2008; Whitham et al., 2007), it is conceivable that passive heating strategies can at least partly mimic the acute inflammatory response observed following exercise. This would have implications for individuals without the physical capacity to engage in sufficient volumes of exercise. Several methods exist to passively increase body temperature in humans, of which sauna bathing and hot water immersion (HWI) are the most commonly used. While these methods are associated with several positive health outcomes, such as weight loss (Hooper, 1999), improved sleep quality (Dorsey et al., 1996) and vascular function (Brunt et al., 2016), research into the potential of passive heating to reduce chronic low-grade inflammation and improve cardiometabolic health is still in its infancy.

Cytokines

Exercise studies suggest that contracting muscle is responsible for the increased circulating concentrations of IL-6 following exercise (Steensberg et al., 2000). Nonetheless, animal studies show that the IL-6 production in skeletal muscle increases following passive heat stress as well (Welc et al., 2012). In a follow-up study, Welc et al. (2013) showed that the upregulation of IL-6 production may be the consequence of HSF-1 activation, which results in an increased production of IL-6, but also iHsp72. Another suggested mechanism for IL-6 release from the muscle in response to hyperthermia is via increased calcium influx after activation of the thermosensitive transient receptor potential 1 (Obi et al., 2016). Although these studies have provided rationale to study HWI in the context of
chronic low-grade inflammation, it should be noted that in animal studies $T_{\text{core}}$ is increased to a much larger extent than considered safe in human participants (Geiger and Gupte, 2011; Silverstein et al., 2014; Welc et al., 2012), which could make HWI less potent to induce an acute inflammatory response in humans. For instance, in the study of Gupte et al. (2011) $T_{\text{core}}$ of the mice was kept between 41°C and 41.5°C for 30 min, while in human studies the maximal attained $T_{\text{core}}$ remained between 38°C and 39°C (Faulkner et al., 2017a; Oehler et al., 2001). Further increases in $T_{\text{core}}$ are associated with considerable health risks in humans (Epstein and Roberts, 2011).

An overview of studies investigating the acute response of inflammatory markers following passive heating is provided in Table 1.1. Despite smaller increases in $T_{\text{core}}$ during passive heating in human compared to animal studies, 1-2 h HWI induces an acute IL-6 response in humans (Faulkner et al., 2017a; Laing et al., 2008; Leicht et al., 2015). Consistent with exercise studies (Fischer, 2006), the acute IL-6 response seems to be dose-dependent. Laing et al. (2008) reported a ~12 fold increase in IL-6 immediately following 2 h HWI in water set at 38.5°C, while 1 h HWI results in a ~2-3 fold increase in plasma IL-6 concentration (Faulkner et al., 2017a; Leicht et al., 2015). Interestingly, HWI induces an acute IL-6 response in people with CSCI of similar magnitude when compared with able-bodied individuals (Leicht et al., 2015). As the IL-6 response following exercise is blunted in people with CSCI (Ogawa et al., 2014), it warrants the question as to what the reason for this discrepancy is. Studies indicating the blunted IL-6 response to exercise in CSCI have not measured $T_{\text{core}}$ (Ogawa et al., 2014; Paulson et al., 2013). It may be, therefore, that the attained $T_{\text{core}}$ during exercise in people with a reduced active muscle mass and physical capacity (i.e. individuals with CSCI) is not high enough to induce a muscle contraction-induced IL-6 response (Rhind et al., 2004). Furthermore, as catecholamines are associated with the IL-6 response following exercise (Paulson et al., 2013) and the increase in plasma catecholamine concentrations following HWI is also limited in able-bodied individuals (Leicht et al., 2015), the mechanisms through which plasma IL-6 concentrations are increased may differ between HWI and exercise.
Table 1.1 The acute effect of passive heating on inflammatory markers.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Design</th>
<th>Main outcome measures</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Faulkner et al., 2017</td>
<td>1 h HWI up to the waist in water set at 40℃</td>
<td>Plasma eHsp72, IL-6 concentration</td>
<td>↑ eHsp72, IL-6</td>
</tr>
<tr>
<td>Leicht et al., 2015</td>
<td>1 h HWI up to the neck in water set 2℃ higher than resting Tcore, able-bodied and CSCI participants</td>
<td>Plasma IL-6, IL-8, IL-1ra concentration</td>
<td>↑ IL-6, IL-1ra → TNF-α</td>
</tr>
<tr>
<td>Oehler et al., 2001</td>
<td>2 h HWI up the neck in water set at 39.5℃</td>
<td>iHsp72 in monocytes</td>
<td>↑ iHsp72</td>
</tr>
<tr>
<td>Morton et al., 2007</td>
<td>1 h HWI of 1 leg in water set at 45℃</td>
<td>iHsp72 in skeletal muscle</td>
<td>→ iHsp72</td>
</tr>
<tr>
<td>Laing et al., 2008</td>
<td>2 h HWI in water set at 38.5℃, control at 35℃</td>
<td>Serum IL-6 concentration</td>
<td>↑ IL-6</td>
</tr>
<tr>
<td>Whitham et al., 2007</td>
<td>2 h HWI in water set at 38.5℃; control at 35℃</td>
<td>Plasma eHsp72 concentration</td>
<td>→ eHsp72 compared with 34℃</td>
</tr>
<tr>
<td>Hafen et al., 2018</td>
<td>2 h heating of skeletal muscle using pulsed wave diathermy</td>
<td>iHsp72 in skeletal muscle</td>
<td>→ iHsp72</td>
</tr>
<tr>
<td>Zychowska et al., 2018</td>
<td>30 min sauna bathing at 98.2℃; athletes and sedentary individuals</td>
<td>Hsp72, IL-6, IL-10 mRNA in leukocytes</td>
<td>→ iHsp72, IL-6, IL-10</td>
</tr>
<tr>
<td>Iguchi et al., 2012</td>
<td>30 min in room set at 73℃</td>
<td>eHsp72</td>
<td>↑ eHsp72</td>
</tr>
</tbody>
</table>

Abbreviations: HWI = hot water immersion; IL = interleukin; IL-1ra = interleukin-1 receptor antagonist; iHsp72 = intracellular heat shock protein 72; eHsp72 = extracellular heat shock protein 72; mRNA = messenger ribonucleic acid

Heat shock protein 72

As mentioned before, passive heating can result in the activation of HSF-1, leading to iHsp72 production (Welc et al., 2012). Passive heating in animal studies indeed results in an acute increase in iHsp72 expression (Gupte et al., 2011; Kavanagh et al., 2016). In vervet monkeys, maintaining T_core between 39℃ and 41℃ for 30 min using HWI results in a significant increase in iHsp72 gene expression in skeletal muscle. In addition, maintaining T_core of mice around 41-41.5℃ for 20 min using a thermal blanket leads to a ~3 fold increase in iHsp72 protein expression in skeletal muscle when compared with control (Gupte et al., 2011). Of note, the acutely increased iHsp72 expression in these 2 studies was also associated with improved insulin sensitivity.
Despite promising evidence from animal studies, results in human studies show mixed results. In fact, only 2 studies have investigated the acute iHsp72 response to HWI in humans and found no increase in skeletal muscle after 1 h (Morton et al., 2007), but an increase in monocyte iHsp72 expression after 2 h HWI (Oehler et al., 2001). The discrepancy between both studies might be caused by the difference in heat exposure as well as the cell type investigated to assess iHsp72 expression. Supporting the suggested importance of significant heat exposure for an acute iHsp72 response, Gibson et al. (2016) showed that Tcore needs to be maintained above 38.5°C for at least 27 min to induce the upregulation of iHsp72 mRNA following exercise. Although the acute iHsp72 response to exercise in total leukocytes has a similar pattern as the response in skeletal muscle (Tuttle et al., 2017), monocytes are particularly responsive to heat stress when compared with other leukocyte subsets (Bachelet et al., 1998). Therefore, the iHsp72 response to HWI may be more pronounced in monocytes when compared with other cell types.

As for the acute iHsp72 response to HWI in humans, not much is known about the potential of HWI to induce an acute increase in eHsp72 concentration. Nonetheless, Faulkner et al. (2017) reported a similar increase in eHsp72 concentration following HWI when compared with exercise matched for heat production. The elevation of muscle temperature was the strongest predictor for the eHsp72 response, explaining 27% of its variance (Faulkner et al., 2017a). Passive heating by 30 min of sauna bathing, resulting in a 0.8°C Tcore increase, also leads to the elevation of eHsp72 concentrations (Iguchi et al., 2012). In contrast, Whitham et al. (2007) found no significant effect of passive heating in water set at 38.5°C when compared with immersion in water set at 35.3°C on the acute eHsp72 response. Therefore, partly due to the lack of a control condition in 2 of the 3 studies, the potential of passive heating to elevate eHsp72 concentrations is equivocal.

**Nitric oxide**

Apart from its impact on markers for chronic low-grade inflammation, another mechanism by which passive heating may exert its beneficial effect on cardiometabolic health is by elevating the bioavailability of nitric oxide (NO) via increased activation of nitric oxide synthase (NOS) in
response to the heat stress and increase in blood flow (Krause et al., 2015b). Nitric oxide is suggested to directly stimulate glucose uptake (Roberts et al., 1997), while its vasodilatory function can result in increased tissue perfusion and therefore delivery of glucose to peripheral tissues (Baron et al., 1994). In support for the importance of NO in glucose metabolism, individuals with impaired insulin sensitivity and T2DM have reduced levels of NO bioavailability (Sansbury and Hill, 2014). In contrast, acute and chronic supplementation with beetroot juice to elevate plasma concentrations of nitrate, a pre-cursor of NO, does not improve glucose metabolism compared to placebo (Fuchs et al., 2016; Gilchrist et al., 2013). Nevertheless, NO inhibition studies suggest that the restoration of NO bioavailability may prove beneficial for glucose metabolism (Sansbury and Hill, 2014). Although passive heat stress indeed results in acute vasodilation (Moyen et al., 2015; Thomas et al., 2017), no direct measures of eNOS activity or NO bioavailability following acute passive heat stress are reported in humans. Future studies investigating the potential of passive heating to increase NO bioavailability in humans could provide insight in yet another mechanism by which this intervention may enhance cardiometabolic health.

1.11 Chronic adaptations to passive heating interventions

Acute studies have confirmed the potential of HWI to induce an inflammatory response (Faulkner et al., 2017a; Laing et al., 2008; Leicht et al., 2015; Oehler et al., 2001), which has led several reviews to suggest that chronic HWI treatment can indeed reduce chronic low-grade inflammation and improve metabolic health (Hooper et al., 2014; Krause et al., 2015b; McCarty et al., 2009; Thomas, 2017). However, there are little human data to support the notion that the acute responses following HWI indeed result in chronic adaptations in the inflammatory profile when regularly engaging in HWI. Nonetheless, animal studies provide some insight in the efficacy of chronic passive heat therapy and the few human studies available show promise.

Most animal studies investigating the impact of chronic passive heat therapy on metabolic health and chronic low-grade inflammation have focussed on basal iHsp72 expression and its impact on insulin sensitivity (Chung et al., 2008; Gupte et al., 2009; Kavanagh et al., 2016; Silverstein et al.,
In mice, 16 weeks of heat therapy results in an increased basal iHsp72 in skeletal muscle in concert with improved insulin sensitivity compared with a sham control condition (Chung et al., 2008). To further support the importance of iHsp72 for insulin sensitivity, elevating iHsp72 expression by pharmacological means or genetic manipulation results in similar improvements in insulin sensitivity (Chung et al., 2008). A simultaneous increase in basal iHsp72 expression and improvement in insulin sensitivity was also reported in the studies of Gupte et al. (2009) and Silverstein et al. (2014). Mechanistically, the link between both adaptations following passive heating seems to involve the inhibition of JNK and NF-κB activation (Henstridge et al., 2014b). Indeed, Chung et al. (2008) and Gupte et al. (2009) both reported reduced activation of these pathways following passive heat therapy. Moreover, in humans, low iHsp72 expression is associated with impaired insulin sensitivity but also elevated JNK activity (Chung et al., 2008).

In a series of studies, Kavanagh et al. (Chichester et al., 2015; Kavanagh et al., 2016, 2011) showed that the elevation of iHsp72 can also lead to beneficial adaptations in a species genetically closer to humans, namely non-human primates. In these animals, 2 HWI sessions per week for 5 weeks increases basal iHsp72 expression in skeletal muscle, which was strongly associated with fasting blood glucose and the insulin responses during an intravenous glucose tolerance test (Kavanagh et al., 2016). In keeping with the data collected in mice (Chung et al., 2008), pharmacological induction of iHsp72 expression also elevates iHsp72 expression and improves insulin sensitivity in non-human primates (Kavanagh et al., 2011). Together, animal studies have highlighted the potential of chronic passive heating interventions to improve the inflammatory profile and metabolic health, possibly via the elevation of basal iHsp72 expression.

An overview of studies that have investigated the effect of passive heating interventions on resting inflammatory and metabolic markers is provided in Table 1.2. No studies on human participants have investigated the potential of chronic whole-body passive heating interventions to elevate basal iHsp72 protein expression and its association with glucose metabolism. Nonetheless, Hooper (1999) showed that 3 weeks of HWI reduces fasting glucose and glycated haemoglobin concentration in people with T2DM, while a reduction in fasting glucose concentration was also
observed following 2 weeks of sauna therapy in patients with congestive heart failure (Biro et al., 2003). Moreover, a 2-week HWI intervention in people with chronic heart failure reduces plasma IL-6 concentrations at rest (Oyama et al., 2013). However, these studies provided little detail on the impact of the HWI sessions on physiological outcome measures such as Tcore and the diseased state of the participants precludes any conclusions regarding healthy (disabled or able-bodied) individuals. In healthy young males, 9 days of passive heat acclimation does not reduce the resting plasma concentration of IL-6 (Kanikowska et al., 2012). However, this could also be due to the relatively modest body temperature increase induced in each session. Moreover, the effect of the acclimation period was assessed on the day after the last passive heating session, possibly resulting in the assessment of acute instead of chronic effects. Although the same concern may apply to Zychowska et al., (2018), 4 weeks of sauna therapy reduced iHsp72 and IL-6 mRNA expression in leukocytes. Interestingly, together these studies suggest that, providing a sufficient thermal load, improvements in markers for glucose metabolism and chronic low-grade inflammation can be made in as little as 2 weeks. As animal studies to induce elevations in basal iHsp72 expression have used longer duration protocols (Chung et al., 2008; Silverstein et al., 2014), this leaves the question whether the observed improvements glucose metabolism reported in humans are indeed orchestrated by the actions of iHsp72.
Table 1.2 The effect of chronic passive heating interventions on inflammatory and metabolic markers.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Design</th>
<th>Main outcome measures</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hooper, 1999</td>
<td>3 weeks HWI with 18 sessions of 30 min in water set between 37.1 and 41℃</td>
<td>Fasting glucose, glycosylated Hb conc.</td>
<td>↓ glucose, glycated Hb</td>
</tr>
<tr>
<td>Oyama et al., 2012</td>
<td>2 weeks HWI with 14 sessions of 10 min in water set at 40℃</td>
<td>Plasma IL-6, CRP, TNF-α conc.</td>
<td>↓ IL-6, CRP, TNF-α</td>
</tr>
<tr>
<td>Kihara et al., 2004</td>
<td>2 weeks of daily sauna bathing for 10 min at 60℃ + blanket for 30 min</td>
<td>Plasma TNF-α conc.</td>
<td>→ TNF-α</td>
</tr>
<tr>
<td>Hafen et al., 2018</td>
<td>6 days of 2 h heating of skeletal muscle using pulsed wave diathermy</td>
<td>iHsp72 in skeletal muscle</td>
<td>↑ iHsp72</td>
</tr>
<tr>
<td>Zychowska et al., 2018</td>
<td>12 sauna baths in 4 weeks for 30 min at 98.2℃</td>
<td>iHsp72, IL-6, IL-10 conc.</td>
<td>↓ iHsp72, IL-6*</td>
</tr>
<tr>
<td>Biro et al., 2003</td>
<td>2 weeks of daily sauna bathing for 15 min at 60℃ + 30 min blanket</td>
<td>Fasting glucose conc.</td>
<td>↓ glucose</td>
</tr>
<tr>
<td>Kanikowska et al., 2012</td>
<td>9 sessions of 10 minHWI in water set at 42℃ + 90 min blanket in 40℃</td>
<td>Plasma IL-6, TNF-α conc.</td>
<td>→ IL-6, TNF-α</td>
</tr>
</tbody>
</table>

Abbreviations: HWI = hot water immersion; Hb = haemoglobin; IL = interleukin; CRP = C-reactive protein; TNF-α = tumour necrosis factor-α; iHsp72 = intracellular heat shock protein 72; mRNA = messenger ribonucleic acid. * A trend for a decreased resting IL-6 mRNA expression

Studies investigating the impact of chronic passive heating interventions on vascular function suggest that passive heating may be a viable method to elevate NO bioavailability in humans (Bailey et al., 2016; Brunt et al., 2016). For instance, 8 weeks of HWI leads to improvements in flow mediated dilation, arterial stiffness, blood pressure (Brunt et al., 2016) and cerebral blood flow (Bailey et al., 2016). Although aforementioned adaptations are possibly mediated by elevations in NO bioavailability (Fürstermann and Sessa, 2012), no direct measures of this marker are made in these studies. In hamsters, on the other hand, 4 weeks of daily passive heating using a far infrared-ray dry sauna increases mRNA and protein expression of eNOS (Ikeda et al., 2005). Appreciating the cross-talk between NO and inflammatory markers (Krause et al., 2015b; Raubenheimer et al., 2017), future studies could combine these biochemical markers with more clinical measures of vascular function.
and glucose metabolism. Collectively, although initial human studies show promise for the use of HWI to improve the inflammatory profile and metabolic health, further research is needed before this strategy can be implemented in practice. For instance, more information on the potential of chronic interventions to alter the basal expression of inflammatory markers such as iHsp72 in humans is needed. Moreover, whether an increased expression of iHsp72 via passive heating also leads to enhanced glycaemic control in humans is currently unclear.

1.12 Combining physiology and psychology - perceptual responses to exercise and related health interventions

Despite the link between acute responses and chronic adaptations to health interventions (Dawson et al., 2018), one must engage regularly in an activity to benefit from its health promoting effects. With currently around 25% of adults worldwide not meeting the physical activity guidelines (World Health Organization, 2017) and drop-out rates in exercise interventions often approaching 50% after the first 6 months (Dishman, 1988), promoting adherence is crucial in the design of health interventions. Exercise adherence is a complex phenomenon, dependent on personal (e.g. self-efficacy), environmental (e.g. accessibility of exercise facilities) and social factors (e.g. peer support) (Rhodes et al., 2009). In addition, a factor that has gained support in the last decades as a possible determinant for future engagement in physical activity are the perceptual responses to and during exercise (Ekkekakis et al., 2011). Most notably, the acute affective responses during exercise (Ekkekakis et al., 2011) and enjoyment of exercise (Ryan et al., 1997) are increasingly recognised to influence exercise adherence.

Affect is described as “a neurophysiological state consciously accessible as simple primitive non-reflective feeling most evident in mood and emotion but always available to consciousness” (Barrett and Bliss-Moreau, 2009). In the context of exercise, this is the pleasure or displeasure people feel while exercising, as assessed with for example the Feeling Scale (FS; Hardy and Rejeski, 1989). Indeed, Williams et al. (2008) showed that the acute affective responses during an incremental exercise protocol predicted exercise behaviour 6 and 12 months later. The concept of enjoyment is
closely related to affect (Russell, 2003). Although there exists no clear consensus on the definition for
enjoyment, it is regarded as a state leading to positive affect and closely associated with feelings of
“flow” (Kimiecik and Harris, 1996) and achievement (Scanlan et al., 1993). Supporting the
importance of enjoyment in exercise participation, Ryan et al. (1997) showed in a prospective study
that enjoyment of exercise but not fitness or health goals was a strong predictor of exercise
participation. While there is now support for the influence of perceptual responses in exercise
adherence (Biddle and Mutrie, 2007), less is known about exercise-related factors that can enhance
these responses.

The strong relationship between exercise intensity and the pleasure that people feel during
exercise suggests that the intensity of exercise may influence exercise adherence rates (Ekkekakis et
al., 2008a). While low-intensity exercise elicits positive affective responses in most people, exercise
above the ventilatory or lactate threshold elicits negative affective responses in most people.
However, at intensities around this threshold there seems to be large intra-individual variation in the
affective response (Ekkekakis et al., 2011), possibly related to genetic (Schutte et al., 2017) and
motivational aspects (Biddle and Mutrie, 2007). Although the negative relationship between exercise
intensity and the affective response has repeatedly been shown (Ekkekakis et al., 2011), this is mainly
tested using continuous exercise. However, the renewed interest in HIIT as a strategy to improve
health in previously sedentary individuals and special populations (Biddle and Batterham, 2015)
requires further understanding in the perceptual responses to interval exercise.

Recent studies investigating the perceptual responses to HIIT suggest that the notion that
lower-intensity exercise leads to more positive perceptual responses might be too simplistic. For
instance, Jung et al. (2014) and Kilpatrick et al. (2015) reported indeed more negative affective
responses during HIIT when compared with continuous moderate-intensity exercise. However, in
both studies ratings of enjoyment as assessed after exercise were more positive in the HIIT trial. Of
note, this finding was corroborated using arm-cranking in people with SCI (Astorino and Thum,
2016). Therefore, despite the high exercise intensities attained and the related negative affective
responses, HIIT may be an additional strategy that could enhance exercise participation via increased
enjoyment. As for passive heating strategies, systematically collected data on the perceptual responses during such activity is lacking. In keeping with the Hedonic theory, stating that people are more likely to repeat behaviour they find pleasurable, optimising perceptual responses alongside physiological responses may be key to successful implementation of this strategy. As passive heating is likely to be most beneficial for populations with a reduced exercise capacity or tolerance, who consequently have been less exposed to the physiological strain induced by exercise, positive perceptual responses may be of particular importance for adherence to this intervention. Therefore, to inform health strategies that are both effective and sustainable, more studies that include physiological and psychological measures simultaneously are needed.

1.13 Conclusions and outlook

Chronic low-grade inflammation is increasingly recognised in the aetiology of chronic diseases, such as T2DM and CVD. While exercise can be effective in reducing chronic low-grade inflammation, the body of literature on the potential of upper-body exercise to do so is limited. For instance, as contracting muscle is an important source of circulating inflammatory markers following exercise, the limited muscle mass involved in upper-body exercise may dampen the acute inflammatory response when compared with lower-body exercise. Although the available literature suggests a limited influence of active muscle mass, it is currently unclear to what extent adaptations in the musculature of upper-body trained individuals affect the acute inflammatory response to this form of exercise. Additionally, disability-related factors such as autonomic dysfunction may hamper the potential of exercise to reduce chronic low-grade inflammation in people with SCI.

To inform studies into alternative or additional health promoting strategies for individuals restricted to be physically active, this introduction has described the importance of IL-6, Hsp72 and the distribution of monocyte subsets as markers for chronic low-grade inflammation and the potential of health interventions to change their expression, both acutely as well as chronically. Mediating the acute inflammatory response, further elevating body temperature during exercise may enhance their health promoting potential. Moreover, the increase in body temperature in the absence of muscle
contraction can induce an acute inflammatory response, suggesting that passive heating strategies may have potential as an alternative or addition to exercise to reduce chronic low-grade inflammation. Although chronic studies in humans are scarce, preliminary data and animal studies show promise, suggesting improvements in metabolic health via elevated iHsp72 expression after chronic passive heating interventions.

In summary, therefore, this thesis investigates possible factors impacting on the acute inflammatory response to exercise as well as the potential of interval upper-body exercise and HWI to serve as an alternative health promoting strategy for individuals restricted to engage in (lower-body) exercise.

1.14 Aims and hypotheses

The main aim and hypothesis of each experimental chapter are given below.

Chapter 2. Exercise modality-specific adaptations; do they lead to a dampened acute inflammatory response to exercise?

Aim: To investigate the influence of chronic modality-specific training adaptation on the acute inflammatory response to upper-and lower-body exercise in individuals trained in either modality.

Hypothesis: Chronic training adaptations in the upper-body dampen the acute inflammatory response to exercise using this modality.

Chapter 3. The acute inflammatory response to endurance exercise in people with a spinal cord injury; the role of autonomic function

Aim: To investigate the influence of autonomic function on the acute inflammatory response to a wheelchair half-marathon in recreational wheelchair athletes.

Hypothesis: The dampened acute inflammatory response in people with CSCI is associated with the autonomic dysfunction present in this group.

Chapter 4. Can intervals enhance the inflammatory response and enjoyment in upper-body exercise?

Aim: To compare the perceptual and inflammatory responses between upper-body HIIT and moderate-intensity exercise in recreationally active males.

Hypothesis: HIIT induces a larger acute inflammatory response compared with moderate-intensity exercise, but results in more negative perceptual responses.
Chapter 5. The acute inflammatory response to hot water immersion in sedentary, overweight adults

*Aim:* To investigate the acute inflammatory response to a HWI session in overweight, sedentary males.

*Hypothesis:* A single HWI session results in elevated plasma IL-6, eHsp72 and nitrite concentrations, as well as an increased iHsp72 expression in monocytes.

Chapter 6. The effect of a chronic hot water immersion intervention on inflammatory and metabolic markers in sedentary, overweight adults

*Aim:* To investigate the effect of a 2-week HWI intervention on resting metabolic and inflammatory markers in overweight, sedentary males.

*Hypothesis:* The chronic HWI intervention results in lowered fasting glucose and insulin concentrations, simultaneously with an increased resting expression of iHsp72.

Chapter 7. The effect of temperature and heat shock protein 72 on the ex-vivo acute inflammatory response in monocytes

*Aim:* To investigate the influence of temperature and eHsp72 on the expression of iHsp72 and IL-6 in monocytes, using an ex-vivo model.

*Hypothesis:* Incubation of whole blood at elevated temperatures increases iHsp72 expression in a step-wise manner, which is further enhanced by incubation with eHsp72.
Exercise modality-specific adaptations; do they lead to a dampened acute inflammatory response to exercise?

Sven P. Hoekstra, Matthew N. Westerman, Flavio Beke, Nicolette C. Bishop, Christof A. Leicht

This chapter is accepted for publication in a slightly modified version with *Applied Physiology, Nutrition, and Metabolism*
2.1 Abstract

**Introduction** While adaptations to a short-term training programme can dampen the acute inflammatory response to exercise, less is known about the influence of chronic modality-specific adaptations to training. This study compared the acute inflammatory response to upper- and lower-body interval exercise in individuals chronically trained in these respective modalities. **Methods** Ninety minutes of interval exercise matched for relative PO on an arm-crank (ARM) and cycle ergometer (LEG) was performed by 8 trained paddlers and 8 trained cyclists. Blood samples were taken pre- and post-exercise. Interleukin-6 (IL-6) concentrations were analysed in plasma, while the expression of intracellular Hsp72 was assessed in monocytes (iHsp72). **Results** Interleukin-6 was increased following both modalities (fold change ARM: 7.23±3.56, *p*<0.001; LEG: 9.03±4.82 *p*<0.001), in both groups (cyclists *p*<0.001; paddlers *p*<0.001), but the increase was smaller in ARM compared with LEG (Time x Modality *p*<0.001). ARM induced a smaller iHsp72 response compared with LEG (fold change ARM: 1.07±0.14, *p*=0.102; LEG: 1.18±0.14, *p*<0.001, Time x Modality *p* = 0.039). Following ARM, iHsp72 expression was increased in the cyclists only (fold change cyclists: 1.12±0.11, *p*=0.018; paddlers: 1.03±0.17, *p*=0.647), while iHsp72 expression following LEG was increased in both groups (fold change cyclists: 1.14±0.15, *p*=0.027; paddlers: 1.22±0.13, *p*<0.001). **Conclusion** The acute inflammatory response to lower-body interval exercise was larger compared with work-matched upper-body interval exercise. Moreover, chronic training adaptations to upper-body exercise dampened the iHsp72 response to exercise using this modality. Therefore, exercise may be less effective in reducing chronic low-grade inflammation in individuals relying on their upper body, such as wheelchair users.
2.2 Introduction

The acute inflammatory response to exercise is suggested to be partly responsible for the protective effects of exercise against a range of chronic diseases, such as T2DM and CVD (Gleeson et al., 2011). It is characterised by an immediate increase in predominantly pro-inflammatory markers such as IL-6 and cHsp72, followed by a longer lasting increase in anti-inflammatory cytokines such as IL-1ra and iHsp72. Furthermore, exercise can alter the proportion of monocyte subsets in the circulation, potentially downregulating the inflammatory potential of monocytes (Gleeson et al., 2011). The amplitude of the acute inflammatory response following exercise is dependent on the intensity and duration of the exercise bout (Pedersen, 2006). In addition, the muscle mass involved in the activity seems to be of influence, suggested by the larger increases in IL-6 following running compared to other sports involving a smaller muscle mass (Fischer, 2006).

Forms of exercise that are characterised by a small active muscle mass are activities using the upper body only, such as arm-cranking, handcycling and wheelchair sports; all activities suited for individuals with an impaired ability to engage in lower-body exercise. Due to the smaller muscle mass as well as the differences in structural and functional characteristics of the arm musculature, the acute inflammatory response to upper-body exercise may be different when compared with lower-body exercise (Fischer, 2006). This may make exercise less effective in reducing chronic low-grade inflammation in wheelchair users, a population at a heightened risk for this condition (Manns et al., 2005). However, while initial studies indeed failed to find an acute increase in inflammatory markers (Bergfors et al., 2005; Hirose et al., 2004), larger volume upper-body exercise can induce acute increases in plasma IL-6, IL-1ra and IL-10 concentrations (Paulson et al., 2015). Moreover, Helge et al. (2011) showed in a whole-body exercise model that the arms release more IL-6 into the circulation compared with the legs; and in a direct comparison between intensity-matched upper-and lower-body endurance exercise at a moderate intensity no differences in the inflammatory response between both modalities were found (Leicht et al., 2016).
Despite evidence for the potential of upper-body exercise to induce a similar acute inflammatory response compared with lower-body exercise (Leicht et al., 2016b), the training status of the exercising muscles may impact on this response (Helge, 2010). Indeed, 8 weeks of endurance training dampens the acute iHsp72 response to an acute bout of exercise until exhaustion in the soleus muscle of mice (Smolka et al., 2000), while a 10-week training intervention dampens the acute IL-6 mRNA response following 3 hours of knee extension endurance exercise in humans (Fischer et al., 2004). Moreover, chronic exercise training can dampen the acute IL-6 response to a strenuous bout of running (Gokhale et al., 2007). Therefore, as previous studies on the acute inflammatory response to upper-body exercise have often included participants unaccustomed to this form of exercise (Leicht et al., 2016; Paulson et al., 2015), the potential of upper-body exercise to induce this beneficial response may be smaller in individuals adapted to this modality.

Since it is now widely acknowledged that skeletal muscle plays a major role in the acute inflammatory response to exercise (Fischer, 2006; Gleeson et al., 2011), local adaptations resulting from exercise training are likely to play a role in the potentially altered acute inflammatory response to exercise following chronic training. Adaptations in the exercising muscle that may affect this response include an increased glycogen content, capacity to oxidise fat (Coffey and Hawley, 2006) and elevated iHsp72 expression (Morton et al., 2009). However, while previous studies suggest an impact of physical fitness on the acute inflammatory response to exercise (Fischer et al., 2004; Gokhale et al., 2007; Smolka et al., 2000), no studies have investigated the influence of exercise modality-specific adaptations to chronic exercise training. As even in sedentary wheelchair users the upper-body musculature is relatively well-trained compared with able-bodied individuals from the general population (Jacobs et al., 2013), such insight may provide more understanding on the potential of upper-body exercise to induce the beneficial acute inflammatory response in people adapted to this form of exercise.

Therefore, to investigate the impact of local training status on the acute inflammatory response to upper- as well as lower-body exercise, this study assessed the acute elevation of circulating inflammatory markers following prolonged interval cycling and arm-cranking in athletes.
specifically trained for either modality. The relatively strenuous character of the exercise bout was chosen to amplify the potential differences in absolute workload between both groups. In addition, a large volume of exercise seems needed to elevate iHsp72 expression in monocytes (Yamada et al., 2008). Finally, intervals were included as this may enhance the enjoyment of exercise (Jung et al., 2014).

2.3 Methods

Eight chronically upper body trained males (i.e. paddlers) and 8 chronically lower body trained males (i.e. cyclists) volunteered to participate in this study. Participants had at least 2 years of experience in their specific sport and reported to train 4 times per week or more. Exclusion criteria were: engagement in more than once a week cross-training (e.g. cycling or running for the paddlers or upper-body strength and conditioning for the cyclists), smoking and the use of anti-inflammatory drugs. After being informed about the procedures of the study, all participants gave written informed consent at the start of the first visit. The study was approved by the local ethics committee of Loughborough University, in accordance with the Declaration of Helsinki.

Study design

Participants visited the laboratory on 4 occasions. In the first 2 preliminary visits, peak exercise capacity was assessed for the upper- as well as lower-body, using a graded incremental exercise test (GXT) on an arm-crank and cycle ergometer, respectively. Thereafter, 2 main trials consisting of a 90 min interval protocol were conducted on the arm-crank (ARM) and cycle ergometer (LEG). The order in which participants performed the GXTs and ARM and LEG was counter-balanced. In the 24 h prior to each visit, participants refrained from strenuous exercise, caffeine and alcohol and standardised their diet using a food diary. Participants consumed a carbohydrate-rich meal before each visit. The main trials all started between 8:00-9:30 am, at the same time for each participant to account for a circadian rhythm in any of the outcome measures.
**Preliminary visits**

On arrival at the laboratory, percentage body fat using 4 site skinfold measurements (Durnin and Womersley, 1973), body mass and height were measured. In addition, leg and arm volume were estimated using the methods described by Katch and Katch (1974) and Brorson et al. (2012), respectively. Briefly, the circumference of the arm and leg was taken at 6 sites, as well as the length between them. This was used to calculate the volume of the resulting truncated cones.

Peak exercise capacity for the upper- and lower body was assessed using a GXT on an arm-crank (Angio, Lode, Groningen, The Netherlands) and cycle ergometer (Excalibur, Lode, Groningen, The Netherlands), respectively. A 5 min warm-up was performed at 5 W (arm-crank ergometer) or 40 W (cycle ergometer) prior to the GXT. Following a 2 min rest, the test started at 5 W (arm-crank ergometer) or 40 W (cycle ergometer), which was increased by 15 W (arm-crank ergometer) or 35 W (cycle ergometer) every 3 min. Cadence was kept in the range between 70-90 rpm for both modalities. Heart rate (HR) was measured continuously throughout the tests (Polar RS400, Kempele, Finland), whilst \( \dot{V}O_2 \) was determined in the last minute of each incremental stage using Douglas bags and a gas analyser (Servomex 1440, Crowborough, UK). Local, central and overall ratings of perceived exertion (differentiated RPE) were reported and capillary blood was taken from the earlobe in the last 30 s of each incremental stage for the assessment of BLa, using a Biosen C-line (Biosen, Barleben, Germany). The tests were terminated at volitional exhaustion or when the requested cadence could no longer be maintained.

Immediately after completion of the tests, RPE was reported and a capillary blood sample was taken. The HR averaged over the last 30 s of the test was defined as \( HR_{peak} \), while the highest \( \dot{V}O_2 \) value during the test was considered \( \dot{V}O_2_{peak} \). The \( PO_{peak} \) was determined as the PO of the last stage fully completed plus the fraction of the stage in which the test was terminated. The GXTs were regarded valid when the respiratory exchange ratio \( \geq 1.10 \) or \( HR_{peak} \geq 95\% \) of the predicted \( HR_{peak} \) for the specific modality in combination with a final RPE \( \geq 19 \). One participant was excluded from participation in the main trials because of 2 non-valid GXTs.
Main trials

An overview of the procedures during the main trials is given in Fig. 2.1. All participants performed 90 min interval exercise on both ARM and LEG. After 10 min of seated rest, a blood sample was taken from an antecubital vein. The exercise session consisted of 10 blocks of 9 min starting with a 3 min stage at 40% \(P_{O_{peak}}\), followed by a 4.75 min stage at 60% \(P_{O_{peak}}\), a 1 min stage at 90% \(P_{O_{peak}}\) and 15 s passive rest. Temperature and humidity during the trials was 21.8±1.1°C and 50±4% for ARM and 21.9±0.9°C and 47±5% for LEG, respectively (\(p>0.431\)).

During the trial, HR and skin temperature at 4 sites (\(T_{skin}\); Ramanathan, 1964) using skin thermistors (I-buttons, Homechip Ltd, Milton Keynes, UK) were measured continuously, while expired air was collected from min 2 until min 3 of the 40%\(P_{O_{peak}}\) and 60%\(P_{O_{peak}}\) stage of block 1, 5 and 9. Capillary blood was taken from the earlobe at the end of those same blocks. Perceptual responses were reported and \(T_{core}\), using a telemetry pill (CorTemp, Palmetto, Florida), was measured at the end of the 40%\(P_{O_{peak}}\), 60%\(P_{O_{peak}}\) and 90%\(P_{O_{peak}}\) stages of block 1, 3, 5, 7 and 9. Participants took the telemetry pill on the evening prior to the trials to avoid the effect of water ingestion on the
temperature readings. The perceptual responses assessed were affect using the Feeling Scale (FS) (Hardy and Rejeski, 1989), thermal sensation (Epstein and Moran, 2006) and differentiated RPE (Paulson et al. 2013). Participants were allowed to drink water *ad libitum*, which was provided using a bladder to avoid differences in the convenience of drinking between ARM and LEG. Nude body mass was measured before and directly following the exercise bout to calculate sweat loss, corrected for the volume of water consumed.

Immediately following completion of the exercise bout, a second blood sample was taken. Thirty minutes following the cessation of exercise, participants filled-out the Physical Activity Enjoyment Scale (PACES) (Kendzierski and DeCarlo, 1991) and reported a session RPE (sRPE)(Foster et al., 2001) of the completed exercise bout. Two hours after completion of the exercise, the last blood sample was taken following 10 minutes of seated rest. Participants were allowed to drink water *ad libitum* but refrained from food in the time between completion of the exercise and the 2 h post blood sample.

**Blood analyses**

Blood was drawn from an antecubital vein into a K$_3$EDTA tube. The blood was spun down immediately for 5 min at 1500 g, and plasma was stored at -80°C until analyses by enzyme linked immunosorbent assay (eHsp72: Amp’d HSP70 high-sensitivity, Enzo life sciences, Farmingdale, US; IL-6: High-sensitivity, RnD systems, Abington, UK). Plasma IL-6 concentrations were only assessed in the pre and immediately post-exercise samples as previous literature indicates that plasma IL-6 concentrations peak immediately after and return to baseline 2 h following exercise similar to the protocol employed in the present study (Fischer, 2006). The intra-plate coefficients of variations (CVs) were 7.1% and 6.4% for eHsp72 and IL-6, respectively. A whole blood count was obtained using a Yumizen H500 cell counter (Horiba Medical, Montpellier, France) for the determination of leukocyte subsets and haemoglobin (Hb), while haematocrit (Hct) was determined using a microcentrifuge. The latter 2 were used to correct the post-exercise plasma IL-6 and eHsp72 concentrations for changes in plasma volume (Dill & Costill, 1974).
Flow cytometry was used to assess changes in iHsp72 in total CD14+ monocytes and the distribution in monocyte subsets. Sixty µl of whole blood was incubated together with 5 µl PerCP-conjugated CD14 and 2.5 µl PE-conjugated CD16 antibodies in the dark at room temperature for 15 min. Thereafter, samples were lysed by Facs lysing solution (BD Biosciences, San Diego, US), washed with phosphate buffered saline (PBS) and fixed using Leucoperm (BD Biosciences). Following permeabilisation (Leucoperm, BD Biosciences) samples were incubated with 4 µl FITC-conjugated Hsp70 antibody or isotype control for 30 min. Finally, samples were washed and resuspended in PBS prior to running through the Flow Calibur (BD Biosciences). The staining procedure was started immediately after blood sample collection and were run through the flow cytometer directly upon completion.

All antibodies except CD16 (BD Biosciences) were purchased from Miltenyi Biotech (Teterow, Germany). Cell Quest software (BD Biosciences) was used for the analysis. Monocytes were selected based on their CD14 expression, the percentage of monocyte subsets (CD14++CD16-classical monocytes, CD14++CD16+ intermediate monocytes and CD14+CD16++ non-classical monocytes) was determined using the trapezoid method (Zawada et al., 2015). The iHsp72 expression in monocytes was determined using the geometric mean fluorescence intensity (GMFI) following subtraction of the isotype control GMFI.

Statistical analysis

Participant characteristics and outcome measures are given in means and standard deviations (SD). When the assumption of normality was violated, identified by the Shapiro Wilk test, data were log transformed before analysis. A Greenhouse-Geisser correction was applied when the assumption of sphericity was violated, which was tested with Mauchley’s sphericity test. For both modalities, a repeated measures ANOVA was used to test for differences between time points and groups, while the same method was used to test for a Time x Modality effect with all participants grouped together. Post-hoc Bonferroni corrected tests were used for further inspection when statistical significance was detected. Differences between the modalities and the groups in personal characteristics and averaged outcome measures of the main trials were assessed using independent and paired sample T-tests,
respectively. Correlations between physiological and inflammatory markers were assessed using Pearson’s \( r \) tests. As the latter was performed as an explorative analysis and a type II error was therefore deemed less problematic, no Bonferroni adjustments were made for significance testing of the correlational analyses (Perneger, 1998). Statistical significance was set at \( p<0.05 \). The 23\textsuperscript{rd} version of the statistical software package SPSS (SPSS inc, Chicago, IL) was used for all analyses.

2.4 Results

Participants

The paddlers and cyclists differed in \( \dot{V}O_2 \text{peak} \) on the arm-crank and cycle ergometer, with higher values for both groups on their respective familiar exercise modality (\( p<0.019 \))(Table 2.1).

Absolute PO and \( \dot{V}O_2 \) was higher during LEG compared with ARM (\( p<0.001 \)). The paddlers and cyclists exercised at a higher absolute PO in their familiar trial compared with the group for whom the exercise modality was unfamiliar (\( p<0.005 \))(Table 2.2).

Table 2.1 Participant descriptives for the paddlers and cyclists.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Paddlers</th>
<th>Cyclists</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>20.3±1.9</td>
<td>27.5±10.1</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>179±5</td>
<td>179±7</td>
</tr>
<tr>
<td>Body mass (kg)</td>
<td>72.4±7.5</td>
<td>68.8±5.8</td>
</tr>
<tr>
<td>Body fat percentage</td>
<td>8.9±1.7</td>
<td>8.5±1.6</td>
</tr>
<tr>
<td>Volume leg (L)</td>
<td>10.6±1.5</td>
<td>9.8±1.7</td>
</tr>
<tr>
<td>Volume arm (L)</td>
<td>3.1±0.8</td>
<td>2.3±0.4*</td>
</tr>
<tr>
<td>Training (h/week)</td>
<td>6.8±3.3</td>
<td>8.6±2.5</td>
</tr>
<tr>
<td>( \text{PO}_{\text{peak}} ) arm-crank ergometer (W)</td>
<td>148±27</td>
<td>116±5*</td>
</tr>
<tr>
<td>( \text{PO}_{\text{peak}} ) cycle ergometer (W)</td>
<td>248±31</td>
<td>338±37*</td>
</tr>
<tr>
<td>( \dot{V}O_2 \text{peak} ) arm-crank ergometer (L/min)</td>
<td>3.38±0.57</td>
<td>2.65±0.29*</td>
</tr>
<tr>
<td>( \dot{V}O_2 \text{peak} ) cycle ergometer (L/min)</td>
<td>3.57±0.42</td>
<td>4.13±0.41*</td>
</tr>
<tr>
<td>( \dot{V}O_2 \text{peak} ) arm-crank ergometer (mL/kg/min)</td>
<td>46.6±6.4</td>
<td>39.3±5.6</td>
</tr>
<tr>
<td>( \dot{V}O_2 \text{peak} ) cycle ergometer (mL/kg/min)</td>
<td>49.6±4.2</td>
<td>60.4±5.4*</td>
</tr>
</tbody>
</table>

Abbreviations: \( \text{PO}_{\text{peak}} \): peak power output; \( \dot{V}O_2 \text{peak} \): peak oxygen uptake; ARM: arm-crank ergometer; LEG: cycle ergometer

* Significant difference between paddlers and cyclists

There were no differences in final BLA between ARM and LEG for either of the groups (\( p>0.539 \)). Fig. 2.2 shows the \( T_{\text{core}} \) during the trials for both groups. For both groups LEG induced a larger increase in \( T_{\text{core}} \) compared with ARM (Time x Modality; \( p<0.001 \), but there was no difference
between both groups during either modality (Time x Group; p>0.152). LEG induced a larger sweat loss compared with ARM (p = 0.003). There was a Modality x Group interaction for sweat loss, with the cyclists sweating more compared with the paddlers during LEG (p = 0.006)(Table 2.2).

Fig. 2.2 Core temperature during the trials for the paddlers and cyclists. Data are presented as mean ± SD. Significantly different from (^) ARM or (*) Pre

Table 2.2 Physiological and perceptual responses to the main trials.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>ARM Paddlers</th>
<th>ARM Cyclists</th>
<th>LEG Paddlers</th>
<th>LEG Cyclists</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\dot{V}O_2) at 40%PO_peak (L/min)</td>
<td>1.29±0.26</td>
<td>1.15±0.20</td>
<td>1.78±0.25*</td>
<td>2.08±0.19*</td>
</tr>
<tr>
<td>(\dot{V}O_2) at 60%PO_peak (L/min)</td>
<td>1.68±0.32</td>
<td>1.45±0.19</td>
<td>2.27±0.34*</td>
<td>2.74±0.22*</td>
</tr>
<tr>
<td>%(\dot{V}O_2) peak at 40%PO_peak</td>
<td>39.7±5.8</td>
<td>43.7±7.7</td>
<td>49.8±3.1*</td>
<td>50.6±3.7*</td>
</tr>
<tr>
<td>%(\dot{V}O_2) peak at 60%PO_peak</td>
<td>51.7±6.6</td>
<td>55.0±7.6</td>
<td>63.2±4.2*</td>
<td>66.8±5.1*</td>
</tr>
<tr>
<td>PO at 40%PO_peak (W)</td>
<td>59±11</td>
<td>46±2</td>
<td>99±13*</td>
<td>135±15*^</td>
</tr>
<tr>
<td>PO at 60%PO_peak (W)</td>
<td>89±16</td>
<td>70±3</td>
<td>149±19*</td>
<td>203±22*^</td>
</tr>
<tr>
<td>PO at 90%PO_peak (W)</td>
<td>133±25</td>
<td>104±4</td>
<td>223±28*</td>
<td>304±33*^</td>
</tr>
<tr>
<td>Mean HR (b/min)</td>
<td>142±9</td>
<td>126±11^</td>
<td>157±9*</td>
<td>158±12*</td>
</tr>
<tr>
<td>Final BLa (mmol/L)</td>
<td>3.1±1.2</td>
<td>3.5±0.7</td>
<td>3.3±1.0</td>
<td>3.5±0.7</td>
</tr>
<tr>
<td>Mean T(\text{skin}) (℃)</td>
<td>32.8±0.8</td>
<td>32.8±0.8</td>
<td>33.3±0.9</td>
<td>32.9±0.9</td>
</tr>
<tr>
<td>Sweat loss (L)</td>
<td>1.2±0.4</td>
<td>1.1±0.4</td>
<td>1.4±0.4</td>
<td>2.0±0.4^</td>
</tr>
<tr>
<td>PACES</td>
<td>93.1±10.6</td>
<td>83.1±23.7</td>
<td>90.3±11.4</td>
<td>86.1±21.9</td>
</tr>
<tr>
<td>Final RPE O (6-20)</td>
<td>16.1±2.5</td>
<td>15.3±2.4</td>
<td>17.3±1.8</td>
<td>16.4±2.0</td>
</tr>
<tr>
<td>sRPE (6-20)</td>
<td>14.9±1.5</td>
<td>14.8±2.2</td>
<td>16.3±1.5</td>
<td>15.4±1.6</td>
</tr>
</tbody>
</table>

Abbreviations: PO: power output; \(\dot{V}O_2\): oxygen uptake; HR: heart rate; BLa: blood lactate; T\(\text{skin}\): mean skin temperature; PACES: physical activity enjoyment scale; RPE O: overall rating of perceived exertion; sRPE: session rating of perceived exertion. Significantly different from (*) other modality or (^) group
Both in the paddlers and cyclists iHsp72 expression was increased following LEG (Time; \( p<0.027 \)). However, iHsp72 expression following ARM was increased in the cyclists only (Time; paddlers \( p = 0.647 \), cyclists \( p = 0.018 \)). However, this did not result in a Time x Group interaction in either of the modalities \( (ARM p = 0.396 \), LEG \( p = 0.175 \). When all participants were grouped together, only LEG induced an increase in iHsp72 expression immediately post-exercise (Time; ARM \( p = 0.100 \), LEG \( p<0.001 \)), resulting in a Modality x Time interaction \( (p = 0.039) \)(Fig. 2.3). At 2 h post-exercise, iHsp72 expression had returned to baseline for both modalities, in both groups (pre versus 2 h post-exercise \( p>0.208 \)).

Interleukin-6 concentrations were elevated in both groups, following both modalities (Time; \( p<0.001 \) in both groups for ARM and LEG). There were no differences in the acute IL-6 response between the groups for either modality \( (Time \times Group; ARM p = 0.113 ; LEG p = 0.480) \)(Fig. 2.3). Plasma IL-6 concentrations increased to a larger extent following LEG compared with ARM when all participants were grouped together \( (Time \times Modality; p<0.001) \).

![Graph](image)

Fig. 2.3 The acute iHsp72 and IL-6 responses following ARM and LEG for the paddlers and cyclists. Lines represent individual participants, while bars represent group means. * Significantly different from pre \( (p<0.05) \).
Neither ARM or LEG induced an increase in eHsp72 concentrations immediately post-exercise (Table 2.3, Time; \( p = 0.339 \)), while there was a trend for a decrease in eHsp72 concentrations following both ARM (Time; \( p = 0.060 \)) and LEG (Time; \( p = 0.069 \)) at 2 h post-exercise. This effect was similar between the paddlers and cyclists (Time x Group; \( p > 0.558 \)) The distribution of the monocyte subsets was altered 2 h post-exercise, with an increase of the percentage of classical monocytes (Time; \( p < 0.001 \)), and a decrease in the percentage of intermediate and non-classical subsets (Time; intermediate monocytes \( p = 0.007 \); non-classical monocytes \( p < 0.001 \)). There were no differences between the groups in either of the modalities (Time x Group; \( p > 0.286 \)), nor was there a difference in the change in monocyte subset distribution between ARM and LEG when all participants were grouped together (Time x Modality; \( p > 0.130 \))(Table 2.3).

Table 2.3 Changes in the distribution of monocyte subsets and eHsp72 following ARM and LEG in the paddlers and cyclists.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Time point</th>
<th>ARM</th>
<th>LEG</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Paddlers</td>
<td>Cyclists</td>
</tr>
<tr>
<td>Classical mon (%)</td>
<td>Pre</td>
<td>91.2±2.8</td>
<td>91.5±2.2</td>
</tr>
<tr>
<td></td>
<td>Post</td>
<td>91.3±1.8</td>
<td>92.5±3.3</td>
</tr>
<tr>
<td></td>
<td>Post 2h</td>
<td>93.2±2.5*</td>
<td>94.6±1.2*</td>
</tr>
<tr>
<td>Intermediate mon (%)</td>
<td>Pre</td>
<td>1.88±0.95</td>
<td>1.78±0.66</td>
</tr>
<tr>
<td></td>
<td>Post</td>
<td>1.79±0.78</td>
<td>1.76±0.93</td>
</tr>
<tr>
<td></td>
<td>Post 2h</td>
<td>1.60±0.57</td>
<td>1.48±0.70</td>
</tr>
<tr>
<td>Non-classical mon (%)</td>
<td>Pre</td>
<td>4.72±2.19</td>
<td>4.55±1.75</td>
</tr>
<tr>
<td></td>
<td>Post</td>
<td>4.82±1.50</td>
<td>4.27±2.48</td>
</tr>
<tr>
<td></td>
<td>Post 2h</td>
<td>3.33±1.64*</td>
<td>2.24±0.65*</td>
</tr>
<tr>
<td>eHsp72 (fold change from pre)</td>
<td>Pre</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>Post</td>
<td>1.22±0.24</td>
<td>1.86±1.24</td>
</tr>
<tr>
<td></td>
<td>Post 2h</td>
<td>0.81±0.97</td>
<td>0.41±0.38</td>
</tr>
</tbody>
</table>

Abbrevations: mon: monocytes; eHsp72: extracellular heat shock protein 72
* Significantly different from Pre
The whole blood count before and after the trials is shown in Table 2.4. For both ARM and LEG there were no differences in the leukocyte responses between the paddlers and cyclists (Time x Group; \( p > 0.115 \)). LEG induced a larger increase in leukocyte (Time x Modality; \( p < 0.001 \)), monocyte (Time x Modality; \( p = 0.009 \)), neutrophil (Time x Modality; \( p < 0.001 \)) and lymphocyte numbers (Time x Modality; \( p < 0.001 \)) compared with ARM when all participants were grouped together. There was a trend for lymphopenia at 2 h post-exercise following LEG but not ARM (Time; ARM \( p = 0.157 \); LEG \( p = 0.067 \)).
Table 2.4. Haematological markers in response to ARM and LEG for the paddlers and cyclists.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>ARM</th>
<th>Cyclists</th>
<th>LEG</th>
<th>Cyclists</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Paddlers</td>
<td>Cyclists</td>
<td>Paddlers</td>
<td>Cyclists</td>
</tr>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
<td>P+2h</td>
<td>Pre</td>
</tr>
<tr>
<td>WBC (10^9/L)</td>
<td>4.83±0.47</td>
<td>7.71±4.45*</td>
<td>7.90±0.61*</td>
<td>4.80±1.66</td>
</tr>
<tr>
<td>Lymphocytes (10^9/L)</td>
<td>1.60±0.32</td>
<td>2.49±0.50*</td>
<td>1.69±0.26</td>
<td>1.67±0.45</td>
</tr>
<tr>
<td>Monocytes (10^9/L)</td>
<td>0.47±0.09</td>
<td>0.74±0.22*</td>
<td>0.62±0.14*</td>
<td>0.48±0.16</td>
</tr>
<tr>
<td>Neutrophils (10^9/L)</td>
<td>2.46±0.48</td>
<td>4.16±1.28*</td>
<td>5.64±1.04*</td>
<td>2.51±1.00</td>
</tr>
<tr>
<td>Hct (%)</td>
<td>45.8±1.2</td>
<td>48.3±1.3*</td>
<td>45.7±1.1</td>
<td>45.1±1.8</td>
</tr>
</tbody>
</table>

Abbreviations: WBC: white blood cells; Hct: haematocrit

* Significantly different from Pre
Perceptual responses

The perceptual responses during the trials for both groups are shown in Fig. 2.4 and Table 2.2. Local, central as well as overall RPE increased during ARM (Time; \( p < 0.001 \)) and LEG (Time; \( p < 0.001 \)) in both groups. During LEG and ARM there were no differences in any of the RPE measures between both groups (Time x Group; \( p > 0.245 \)). When all participants were grouped together, there were no differences in any of the RPE measures between ARM and LEG (Time x Modality; \( p > 0.330 \)). There was also no difference between the groups in the sRPE reported following ARM (\( p = 0.914 \)) and LEG (\( p = 0.277 \)), nor was there a difference between both modalities when all participants were grouped together (\( p = 0.189 \)).

During ARM and LEG, basic affect decreased throughout both exercise trials (Time; \( p = 0.005 \)), resulting in lower scores on the FS in block 9 compared with block 1 during 40\%, 60\% and 90\% \( P_{\text{O}_{2\text{peak}}} \) (\( p < 0.036 \)). This progression was similar between the paddlers and cyclists in both modalities (Time x Group; \( p > 0.253 \)). Ratings of enjoyment after both ARM or LEG did not differ between the paddlers and cyclists (Table 2.2; \( p > 0.294 \)).
Correlations

There was a significant correlation between $T_{core}$ attained at the end of the exercise bout and the IL-6 response for both modalities ($T_{core}$ final ARM - $\Delta$IL-6 ARM: $r = 0.54$, $p = 0.04$; $T_{core}$ final LEG - $\Delta$IL-6 LEG: $r = 0.58$, $p = 0.03$). This association with final $T_{core}$ was not present for the acute iHsp72 response following exercise ($T_{core}$ final ARM - $\Delta$Ihsp72 ARM: $r = 0.23$, $p = 0.41$; $T_{core}$ final LEG - $\Delta$Ihsp72 LEG: $r = 0.39$, $p = 0.13$). There was no significant correlation between the acute iHsp72 and IL-6 response ($\Delta$pre – immediately post-exercise) following ARM nor LEG ($\Delta$Ihsp72 - $\Delta$IL-6 ARM: $r = 0.05$, $p = 0.85$; $\Delta$Ihsp72 - $\Delta$IL-6 LEG: $r = -0.04$, $p = 0.88$). Furthermore, there was no significant correlation between $PO_{peak}$ and the iHsp72 or IL-6 response for either of the 2 modalities ($PO_{peak}$ ARM - $\Delta$Ihsp72 ARM: $r = -0.37$, $p = 0.15$; $PO_{peak}$ LEG - $\Delta$Ihsp72 LEG: $r = -0.26$,
\( p = 0.33; \) PO\textsubscript{peak} ARM - \( \Delta \)IL-6 ARM: \( r = -0.38, p = 0.14 \); PO\textsubscript{peak} LEG - \( \Delta \)IL-6 LEG: \( r = 0.03, p = 0.93 \).

2.5 Discussion

This study showed that, although there was no significant Time x Group interaction, chronic upper-body training may attenuate the acute iHsp72 response to a bout of upper-body interval exercise. However, chronic training does not influence the acute IL-6 or eHsp72 response, nor does it influence the change in monocyte subset distribution following exercise. While several studies have reported a dampened acute inflammatory response to exercise in physically fit or chronically trained individuals (Fischer et al., 2004; Gokhale et al., 2007), the findings of the present study indicate that modality- specific adaptations also impact on these acute responses to exercise. This has implications for the interpretation of research findings gathered from cycling studies and upper-body exercise studies using able-bodied, active individuals unaccustomed to upper-body exercise as well as for the creation of exercise programmes for individuals accustomed to upper-body exercise (e.g. wheelchair users).

The attenuation of the acute iHsp72 response to upper-body exercise in individuals adapted to this form of exercise is in accordance with exercise intervention studies in mice. For instance, Smolka et al. (2000) reported an attenuated acute increase in iHsp72 expression in soleus muscle of mice who were trained for eight weeks compared with sedentary control mice following treadmill running. The attenuated iHsp72 response was accompanied by an increase in anti-oxidative enzymes and an attenuated increase in reactive oxygen species following the exercise bout, suggesting that the dampened oxidative stress response following exercise in the trained mice reduces the need for an iHsp72 response. Although oxidative stress was not measured in the present study, this may have mediated the finding in the current study as also in humans short- (Fisher et al., 2011) and long-term (Falone et al., 2010) exercise training can attenuate the acute oxidative stress response following exercise. Additionally, chronic training elevates skeletal muscle glycogen content at rest and reduces glycogenolysis during exercise for a given relative intensity (Hickner, 1997). As glycogen depletion
can impact on the acute iHsp72 response to exercise (Febbraio et al., 2002), this may be an additional explanation for the dampened iHsp72 response following ARM in the paddlers. The absence of a dampened iHsp72 response in the cyclists following LEG may be explained by the fact that humans rely on their legs for locomotion and the difference in adaptations in the legs between the paddlers and cyclists may not have been as pronounced as in the upper body.

As acute IL-6 and iHsp72 elevations are partly induced by similar stressors (e.g. hyperthermia, oxidative stress, acidosis; (Welc et al., 2012)), it may be somewhat surprising that there was no difference in the IL-6 response between both groups for either modality. Indeed, exercise intervention studies have reported an attenuation of the acute IL-6 response following the training period (Croft et al., 2009; Yfanti et al., 2012), while chronically trained athletes also show a dampened acute IL-6 response compared with sedentary individuals (Gokhale et al., 2007). It is possible, however, that the measurement of systemic IL-6 (i.e. plasma concentration) is not sensitive enough to detect any differences in the acute response to exercise resulting from local adaptations in the exercising muscle of the cyclists and paddlers of the present study. For instance, while Fischer et al. (2004) found a markedly attenuated acute IL-6 mRNA response in skeletal muscle following the 10-week intervention period, no difference was observed in plasma IL-6 concentrations. Interestingly, for both ARM and LEG there was a significant correlation between final Tcore and the acute IL-6 response, suggesting that the elevation of plasma IL-6 concentrations following exercise are indeed influenced by more than contraction related processes only. As hyperthermia alone can induce elevations in plasma IL-6 concentrations (Laing et al., 2008), the elevated circulating IL-6 concentration following the exercise bouts may therefore have also originated from other sites than the exercising muscle groups.

In the present study both groups exercised at the same relative intensity during both exercise modalities (i.e. similar %POpeak and %VO2peak between paddlers and cyclists during both ARM and LEG). Therefore, the attenuated iHsp72 response following ARM in the paddlers is not the result of a reduced relative workload compared with the cyclists. In contrast, the attenuation of the acute inflammatory response reported following training interventions in previous studies could partly be
explained by a reduced relative workload, as an identical pre- and post-intervention acute exercise trial was employed and physical fitness was improved as a result of the intervention (Croft et al., 2009; Smolka et al., 2000). Moreover, the opposite effect is present after 2 weeks of immobilisation: an amplified acute IL-6 response can be observed following exercise at a higher relative intensity due to the decrease in physical capacity of the immobilised leg (Reihmane et al., 2013). Therefore, the present study extends on these findings by showing, in corroboration with studies on the effect of physical fitness per se (Gokhale et al., 2007; Yfanti et al., 2012), that chronic modality-specific adaptations to exercise can also attenuate the acute inflammatory response to exercise when performed at the same relative intensity.

Regardless of specific training status, LEG induced a larger acute iHsp72 and IL-6 response when compared with relative intensity-matched ARM. This contrasts earlier studies that have found a similar acute IL-6 response after 45 min of cycling and arm-cranking at 60% $\dot{V}O_2$peak (Leicht et al., 2016) and a similar contribution of the arms and legs to circulating levels of IL-6 during whole-body exercise (Helge et al., 2011). The authors of both studies suggested that the similar IL-6 production from the limbs with a significantly smaller muscle mass (i.e. the arms) may have been the result of the difference in training status between the upper- and lower- body extremities in the recreationally (lower-body) trained participants. This was supported by a difference in citrate synthase activity between both limbs (Helge et al., 2011). Therefore, arm-cranking may have imposed more stress on the exercising muscle than cycling, despite the similar relative workload between both modalities (Leicht et al., 2016b). However, although citrate synthase activity was not measured in the current study, the similar IL-6 response following ARM in the paddlers and cyclists does suggest that the limb-specific training status has not influenced the results of the aforementioned studies.

It should be noted that because of its intermittent character the exercise in the present study was prescribed relative to POpeak. This was chosen as regulating $\dot{V}O_2$ during intermittent exercise is problematic due to the time needed to reach a steady-state $\dot{V}O_2$ during exercise (Whipp and Wasserman, 1972). Therefore, $\dot{V}O_2$ was not controlled and the relative $\dot{V}O_2$ was in fact smaller during ARM compared with LEG. Thus, in contrast to the comparison between the cyclists and paddlers, the
relative internal workload (i.e. \%\(\text{VO}_2\text{peak}\), as opposed to \%\(\text{PO}_2\text{peak}\) as a measure of external workload) differed between both modalities. This did, however, not result in a difference in BLa or RPE between both modalities.

Although the higher relative internal workload during LEG compared with ARM may have mediated the difference in the acute inflammatory response between both modalities (Croft et al., 2009; Smolka et al., 2000), other factors might further explain why the findings of the present study are in contrast with previous studies that have compared upper- and lower-body exercise (Helge et al., 2011; Leicht et al., 2016). For instance, exercise in the study of Leicht et al. (2016) comprised of 45 min of moderate intensity cycling or arm-cranking, which is not likely to induce large elevations in T\(_{\text{core}}\) in either modality. The relatively intense and prolonged interval protocol employed in the present study may have exacerbated the differences in T\(_{\text{core}}\) and related physiological stressors (e.g. dehydration) between upper- and lower-body exercise, resulting in a larger acute inflammatory response following LEG compared with ARM. The significant correlation between T\(_{\text{core}}\) and the acute IL-6 response after both ARM and LEG suggests that the difference in T\(_{\text{core}}\) between both modalities may indeed have partly led to the larger IL-6 response following LEG. Therefore, it may be conceivable that when exercise is more prolonged and performed at a high intensity, upper-body exercise may be less effective than lower-body exercise in inducing an acute inflammatory response.

Despite the difference in relative internal workload between ARM and LEG, there was no difference between both modalities in the acute eHsp72 response. The failure of the exercise protocol employed in the present study to induce acute elevations in eHsp72 may be explained by the relatively modest increases in T\(_{\text{core}}\), with the peak T\(_{\text{core}}\) during LEG being \(\sim 38.8^\circ\text{C}\) and ARM \(\sim 37.7^\circ\text{C}\). For instance, investigating the minimum endogenous heat exposure needed to induce an eHsp72 response with exercise, Gibson et al. (Gibson et al., 2014) concluded that a peak T\(_{\text{core}}\) of 39.2\(^\circ\text{C}\) and a mean T\(_{\text{core}}\) of 38.6\(^\circ\text{C}\) for at least 56 min are needed to induce elevations in eHsp72 concentrations. This may make upper-body exercise, but also lower-body exercise in non-athletic populations an ineffective strategy to induce acute changes in eHsp72 concentrations.
While knowledge on the acute inflammatory response to exercise may aid in the creation of more effective exercise strategies to promote cardiometabolic health, such interventions can only be successful when sufficiently adhered to in the long-term. The perceptual responses to exercise are suggested to be important for exercise adherence (Ekkekakis et al., 2011). Although physical fitness is positively associated with favourable perceptual responses during an acute bout of exercise (Frazão et al., 2016), the present study shows that familiarity to a specific exercise modality does not influence the acute affective response to or enjoyment of exercise. Therefore, a history of training or physical fitness per se may be more important than familiarity to a specific exercise modality for the perceptual responses during exercise. Consequently, having to change between modalities, for instance due to injury, does not necessarily lead to more negative perceptual responses during unaccustomed exercise. This may facilitate adherence to rehabilitation programmes of injured athletes or in people that are forced to change exercise modality due to sudden disability.

Some limitations of this study need mentioning. First, while iHsp72 expression following ARM was increased in the group unaccustomed to upper-body exercise only, no significant Time x Group interaction was observed for this modality, potentially due to inadequate statistical power resulting from the relatively small sample size. Therefore, although this study provides support for the influence of local training status on the acute inflammatory response to upper-body exercise, future studies including a larger number of participants may be needed to confirm this finding. In line with this, although not statistically significant, the cyclists reported to engage in more hours of weekly training compared with the paddlers. Although no iHsp72 response was observed after ARM in the paddlers of the present study, the inclusion of paddlers engaging in larger weekly training volumes could have resulted in a more pronounced difference in the acute increase of inflammatory markers between both groups following ARM. Finally, although participants consumed a carbohydrate-rich meal prior to all trials and food diaries were used to standardise participants’ diet throughout the study, the provision of food to the participants could have mitigated any potential difference in pre-trial meals between both groups.
In summary, the acute inflammatory response to upper-body exercise is smaller when compared with lower-body exercise matched for relative PO. Moreover, chronic upper-body exercise training may further attenuate the acute iHsp72 response to upper-body exercise. Since the acute inflammatory response to exercise is suggested to induce a range of health-promoting adaptations, including improvements in insulin sensitivity and vascular health, the attenuated acute iHsp72 response to upper-body exercise in people adapted to this modality may dampen the beneficial effects of exercise in these individuals. This might warrant additional strategies to maintain or improve cardiometabolic health in wheelchair users, a population that is at an increased risk for chronic low-grade inflammation and related chronic diseases.
The acute inflammatory response to endurance exercise in people with a spinal cord injury; the role of autonomic function

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This chapter is under review in a slightly modified version with The Journal of Sport Sciences.
3.1 Abstract

Introduction The dampened acute inflammatory response to exercise in people with a spinal cord injury (SCI) is suggested to result from the autonomic dysfunction found in this population. This study investigated the relationship between autonomic function assessed at rest as well as in response to exercise, and the inflammatory response to a wheelchair half-marathon in people with SCI.

Methods Seventeen wheelchair athletes with a cervical spinal cord injury (CSCI, N=7) and without a cervical spinal cord injury (NON-CSCI, N=10) participated in a wheelchair half-marathon. Blood was taken prior, post and 1 h post-race to determine the concentrations of adrenaline, noradrenaline, extracellular heat shock protein 72 (eHsp72) and interleukin-6 (IL-6). A sit-up tilt test was performed to assess autonomic function at rest. Results CSCI showed a lower supine ratio of the low and high frequency power of the variability in RR intervals ($p=0.038$), total and low frequency power of the systolic blood pressure variability ($p<0.001$ and $p=0.005$, respectively) compared with NON-CSCI. Following the race, catecholamine concentrations increased only in NON-CSCI (fold increase; NON-CSCI: 4.23±4.31, CSCI: 1.64±1.06, $p<0.036$). The increase in IL-6 post-race was larger in NON-CSCI (fold increase; NON-CSCI: 4.93±2.51, CSCI: 2.26±1.05, $p=0.040$). Post-race catecholamine levels explained 60% of the variance in the IL-6 response ($r=0.77$, $p=0.040$), which was further increased when the resting autonomic function indices were added to the regression model ($R^2>81\%$, $p<0.012$). Conclusion The dampened acute inflammatory response to a wheelchair half-marathon in CSCI was strongly associated with the autonomic dysfunction present in this group.
3.2 Introduction

Chronic low-grade inflammation is more prevalent in people with SCI compared to members of the able-bodied population (Bauman & Spungen, 2008). This might be the consequence of a physically inactive lifestyle and the physiological changes accompanying the injury (Martin Ginis et al., 2010). Exercise training is widely recognised as a means to combat chronic low-grade inflammation (Petersen & Pedersen, 2005), partly as a result of the acute inflammatory response induced by each bout. During and shortly after exercise, the primary source of circulating IL-6 is skeletal muscle (Steensberg et al., 2000). Despite a smaller active muscle mass involved, upper-body exercise has been shown effective in the induction of an acute inflammatory response (Sasaki et al., 2014; Umemoto et al., 2011). However, this response might be attenuated in people with CSCI (Paulson et al., 2013).

Persons with CSCI have a smaller muscle mass and impaired autonomic function compared to able-bodied individuals and people with paraplegia, both impacting on their exercise capacity (West et al., 2015). There is limited research into the influence of these factors on the acute inflammatory response to exercise. Paulson et al. (2013) reported an attenuated IL-6 response to a strenuous bout of exercise in CSCI compared to SCI; a finding that was replicated in a wheelchair racing setting (Ogawa et al., 2014). Together with the attenuated IL-6 response, both studies reported a blunted adrenaline and noradrenaline response to the exercise bout in the CSCI group (Ogawa et al., 2014; Paulson et al., 2013), suggesting a major role of catecholamines in the dampened acute inflammatory response to exercise present in people with CSCI. Indeed, adrenaline infusion in resting able-bodied individuals results in an increased plasma IL-6 concentration (Sondergaard et al., 2000; Steensberg et al., 2001), potentially via the increase of intracellular cyclic adenosine monophosphate (cAMP) following the stimulation of β receptors on skeletal muscle with adrenaline (Sondergaard et al., 2000).

Apart from resulting in lower resting and exercise-induced plasma catecholamine concentrations, CSCI can lead to the disruption of sympathetic innervation of the heart, as well as the skin and blood vessels below the lesion (Krassioukov, 2009). Consequently, members of this
population have an impaired blood pressure regulation and HR attained during maximal exercise does often not exceed ~130 bpm (West et al., 2015). Therefore, although an attenuated catecholamine response to exercise is a characteristic of autonomic dysfunction, the latter affects a plethora of other physiological processes that may influence the acute inflammatory response to exercise. As autonomic completeness of a spinal injury varies between people as a result of the potential sparing of autonomic fibres below the lesion (Krassioukov, 2009), the impact of autonomic dysfunction on the inflammatory response to exercise may also differ between people with SCI.

For a more comprehensive assessment of autonomic function than using exercise-induced plasma catecholamine concentrations alone (Paulson et al., 2013; Ogawa et al., 2014), additional tests exist (Krassioukov, 2009). The sit-up tilt test, for instance, can be used to detect abnormal changes in HR and blood pressure in response to a passive tilt manoeuvre (Claydon & Krassioukov, 2008). Moreover, Claydon & Krassioukov (2008) reported that frequency blood pressure and heart rate variability measures taken in a supine position are predictive of a range of clinical measures of autonomic dysfunction, such as for instance orthostatic hypotension.

The present study therefore extended on previous research (Ogawa et al., 2014; Paulson et al., 2013) by incorporating autonomic function indices measured at rest in addition to the assessment of the catecholamine response following exercise to investigate the association of autonomic function with the inflammatory response to a wheelchair half-marathon. In addition, this study investigated the potential of upper-body exercise in SCI to acutely elevate eHsp72 concentrations, a relatively novel marker implicated in chronic low-grade inflammation (Johnson & Fleshner, 2006). Together, this can provide further insight into factors that influence the acute inflammatory response to exercise and inform strategies to reduce chronic low-grade inflammation in people with CSCI.

3.3 Methods

Participants were 17 male recreational wheelchair athletes with CSCI (N=7) or without CSCI (NON-CSCI, N=10), the latter group including individuals with a spinal lesion below the cervical level (N=6), spina bifida (N=2), polio (N=1) and myotonia congenita (N=1) (Table 3.1). Participants
took part in the wheelchair half-marathon of Oita 2016. The study was approved by the local ethics committees of Loughborough University (United Kingdom) and Wakayama University (Japan) and participants gave informed consent prior to participation.

Table 3.1 Characteristics of the participants with (CSCI) compared to the participants without (NON-CSCI) a cervical spinal cord injury.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>NON-CSCI (N=10)</th>
<th>CSCI (N=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>43.5 (12.1)</td>
<td>43.4 (15.0)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>155.3 (23.3)</td>
<td>172.6 (7.8)</td>
</tr>
<tr>
<td>Body mass (kg)</td>
<td>53.8 (11.4)</td>
<td>60.0 (15.8)</td>
</tr>
<tr>
<td>Wheelchair racing experience (yrs)</td>
<td>15.1 (9.6)</td>
<td>16.3 (10.2)</td>
</tr>
<tr>
<td>Training per week (min)</td>
<td>313 (245)</td>
<td>307 (193)</td>
</tr>
<tr>
<td>Lesion level SCI</td>
<td>&lt;Thoracic 5</td>
<td>&gt;Cervical 8</td>
</tr>
<tr>
<td>Sensory and motor complete/incomplete SCI</td>
<td>N=4/2</td>
<td>N=3/4</td>
</tr>
<tr>
<td>Orthostatic hypotension</td>
<td>N=1</td>
<td>N=4</td>
</tr>
<tr>
<td>Change in SBP/DBP following tilt (mmHg)</td>
<td>-2 (12) / -4 (7)</td>
<td>-21 (10)/-5 (11)</td>
</tr>
<tr>
<td>Supine LF/HF RRI</td>
<td>1.65 (1.22)</td>
<td>0.34 (0.20)*</td>
</tr>
<tr>
<td>Supine LF SBP (Hz)</td>
<td>7.54 (3.71)</td>
<td>1.39 (1.95)*</td>
</tr>
<tr>
<td>Supine TP SBP (Hz)</td>
<td>5.96(3.74)</td>
<td>36.34(11.27)*</td>
</tr>
</tbody>
</table>

Abbreviations: SBP = systolic blood pressure; DBP = diastolic blood pressure; HR = heart rate; LF/HF RRI = ratio of the power in the low and high frequency domain of the heart rate variability; LF SBP = power in the low frequency domain of systolic blood pressure variability; TP SBP = total power of systolic blood pressure variability. Orthostatic hypotension is defined as a drop in SBP>20 mmHg or DBP>10 mmHg following the onset of the sitting position. * Significant difference between NON-CSCI and CSCI (p<0.05).

**Sit-up tilt test**

One to 2 days prior to the race, a sit-up tilt test was conducted in 16 of the 17 athletes for the assessment of autonomic function. All tests were performed using the same wheelchair with adjustable back rest and in a room set at 25°C. Participants were rested for 5 minutes in a supine position and were then elevated into the sitting position, which they maintained for another 5 minutes. Participants were instructed to breathe at a frequency of 0.25 Hz. Blood pressure was measured beat-by-beat at the wrist (MUB101-50, MediSense Inc., Tokyo, Japan) whilst a brachial blood pressure cuff (STBP-780, Colin, Komaki, Japan) was used for the calibration. Both cuffs were situated at the level of the heart for the full duration of the test. Heart rate was continuously monitored using a 7-lead
electrocardiogram (PhysioFlow Lab-1, Manatec Biomedical, Paris, France). Following the test, participants reported their height, body mass, wheelchair racing experience and average time spent training.

To obtain HR and blood pressure variability measures in the frequency domain, beat-to-beat mean arterial pressure and HR were obtained by integrating the respective signals within each cardiac cycle. Cardiac cycles were determined based on the diastolic intervals of the beat-to-beat blood pressure signal. Beat-to-beat mean arterial pressure and HR were first linearly interpolated and resampled at 2Hz and then detrended by subtracting their third polynomial. Subsequently, the beat-to-beat time series were used for the spectral analyses based on the Welch algorithm. Each time series was subdivided into successive 256-point Hann windows that overlapped by 50% before fast Fourier transform analysis (Van der Scheer et al., 2018). Outcome measures used to reflect autonomic function were: the largest drop in blood pressure following the onset of the sitting position (to detect orthostatic hypotension (OH), defined as a drop in SBP>20 mmHg or DBP>10 mmHg (Freeman et al., 2011)), the ratio of the power in the low (power = 0.07 – 0.20 Hz) and high frequency (power = 0.20 – 35 Hz) domain of the heart rate variability (LF/HF RRI), total power and power in the low frequency domain of systolic blood pressure variability (TP SBP and LF SBP, respectively) in the supine position (Claydon & Krassioukov, 2008). These variability measures were chosen as they have been shown reliable in people with SCI (Ditor et al., 2005) and to strongly correlate with clinical symptoms of autonomic dysfunction (Claydon & Krassioukov, 2008).

Wheelchair half-marathon

The Oita wheelchair half-marathon is a 21.1 km race on relatively flat terrain in the city centre. The race started at 10am in relatively mild conditions (23°C, 56% relative humidity). Participants had refrained from exercise in the 24 hours prior to the race. While food and liquid ingestion were not controlled due to the potential interference with the race practices of the athletes, these were reported using a food diary. All participants reported to have consumed a carbohydrate-rich breakfast in the morning of the race and to have consumed a sports drink during the race. Heart rate was continuously measured using a Garmin monitor (Garmin Edge 500, US). Blood was drawn
from an antecubital vein prior to the warm-up for the race (pre), directly after finishing the race (post) and 1 h post-race (1 h post) into a glass serum separation and a K3EDTA tube of which respectively serum and plasma were extracted and stored at -80 °C until analysis. These time-points were chosen based on previous studies on the acute inflammatory response to endurance-type exercise in able-bodied (Fehrenbach et al., 2005; Fischer, 2006) and individuals with disability (Ogawa et al., 2014). Interleukin-6 (High sensitivity; R&D systems, UK) and eHsp72 (Amp’d high sensitivity; Enzo life sciences, US) were analysed in serum using a quantitative sandwich-type enzyme-linked immunosorbent assay (CV: 8.2% and 6.3%, respectively), while adrenaline and noradrenaline were analysed in plasma by high-performance liquid chromatography. Haemoglobin and haematocrit were determined using an automated cell counter (MEK-6400, Nihon Koden, Tokyo, Japan) and were used to correct the outcome markers for changes in plasma volume resulting from the race (Dill and Costil, 1964).

**Statistical analyses**

The participants were divided into 2 groups on the basis of lesion level (regardless of completeness), resulting in a CSCI and NON-CSCI group. All data were checked for normality with the Shapiro-Wilk test, after which the data were log-transformed when this assumption was violated. Data were checked for sphericity using Levene’s test. Comparisons of athlete characteristics and responses to the sit-up tilt test between CSCI and NON-CSCI were assessed using independent student T-tests. Two-way repeated measures ANOVAs were used to assess changes in IL-6, eHsp72, adrenaline and noradrenaline in CSCI and NON-CSCI following the race.

To further assess the influence of catecholamines and the sit-up tilt indices on the inflammatory response following the race, (multiple) linear regression analysis using the whole sample was performed. Adrenaline and noradrenaline were entered into the model both together and individually and the $R^2$ was used to describe the explained variance of the dependent variable. The same was done for LF/HF RRI, TP SBP and LF SBP. Finally, both catecholamines, LF/HF RRI and one of the blood pressure variability measures were entered into the regression model and the change
in R² as compared to the model with the catecholamines or autonomic function indices at rest only was tested using ANOVA. Collinearity among the predictor variables was tested using Tolerance and the Condition Index, whereby values higher than 1 and 15 respectively were considered concerning (Midi et al., 2010). All analyses were performed using the 23rd version of SPSS and statistical significance was defined as $p<0.05$.

3.4 Results

**Sit-up tilt test**

Five participants demonstrated OH in response to the tilt manoeuvre, 4 of which were in the CSCI group and 1 in NON-CSCI. Athletes in the CSCI group showed a larger drop in SBP in response to the tilt manoeuvre compared to NON-CSCI ($p=0.017$). LF/HF RRI ($p=0.038$), TP SBP ($p<0.001$) and LF SBP ($p=0.005$) differed significantly between the 2 groups, with NON-CSCI showing larger values (Table 3.1).

**Wheelchair half-marathon**

At rest, CSCI had lower levels of adrenaline ($p=0.003$) and noradrenaline ($p<0.001$) compared to NON-CSCI. While there was no difference in baseline IL-6 plasma concentrations ($p=0.519$), a strong trend for higher resting eHsp72 concentrations for CSCI compared to NON-CSCI was present ($p=0.055$)(Fig. 3.1).

The NON-CSCI group took significantly less time to complete the race than the CSCI athletes (1.06±0.21 versus 1.43±0.38 h, $p=0.026$). The average HR during the race was significantly higher for NON-SCI compared to CSCI (145±32 versus 103±20 bpm, $p=0.029$). There was a trend for a higher peak HR in the NON-CSCI group (166±41 versus 129±1 bpm, $p=0.087$).

Following the race, serum adrenaline and noradrenaline concentrations were increased in NON-CSCI ($p<0.036$), but not in CSCI ($p>0.113$). The increase in serum IL-6 concentrations in response to the race was larger in NON-CSCI compared to CSCI ($p=0.040$), although the latter group showed a significant increase in IL-6 as well ($p=0.033$). Extracellular heat shock protein 72
did not increase in either of the groups (NON-CSCI: \( p = 0.338 \) and CSCI: \( p = 0.116 \)) (Fig. 3.1). Since both IL-6 and eHsp72 concentrations did not change from post to 1 h post (\( p > 0.391 \)), further analyses were performed using the serum concentrations of both markers immediately following the race only.

**Association between autonomic function and the inflammatory response**

The relationship between the autonomic function indices, catecholamines and the inflammatory response to the race is illustrated in Table 3.2 and Figure 3.2. Post-race plasma concentrations of adrenaline and noradrenaline combined explained 60% of the variance in the IL-6 concentrations following to the race (\( p = 0.04 \)), while adrenaline alone explained 44% of the variance (\( p = 0.007 \)). The LF/HF RRI and TP SBP both individually explained a significant proportion of the variance in post-race plasma adrenaline concentrations (29% and 33%, respectively; \( p < 0.042 \)). The model including combination of LF/HF RRI with LF SBP or TP SBP did not significantly explain the adrenaline response (\( p > 0.08 \)). The variance in the IL-6 response could not be explained by one of the
autonomic function indices in isolation (LF/HF RRI: $R^2 = 8\%$, $p=0.37$; LF SBP: $R^2 = 12\%$, $p=0.27$; TP SBP: $R^2 = 29\%$, $p=0.07$). The same was true for the combination of LF/HF RRI together with either of the 2 variability measures of systolic blood pressure ($p>0.281$). The regression model including adrenaline, noradrenaline, LF/HF RRI and one of the systolic blood pressure variability measures (i.e. LF SBP or TP SBP) increased the explained variance of post-race IL-6 concentrations in comparison to the model including catecholamines only ($R^2>81\%$, $p<0.012$). The change in $R^2$ compared with the model including catecholamines only was significant (adrenaline, noradrenaline, LF/HF RRI, TP SBP; $p=0.036$), or showed a trend towards significance (adrenaline, noradrenaline, LF/HF RRI, LF SBP; $p=0.07$). Of note, Tolerance and the Condition Index were <0.54 and <8.87, respectively, indicating no substantial collinearity among the predictor variables. Finally, athletes that presented with OH during the sit-up tilt test showed lower levels of adrenaline (104 (131) mmol/L versus 265 (243) mmol/L) and IL-6 (4.34 (1.08) pg/ml versus 10.21 (6.84) pg/ml) post-race, although only the difference in IL-6 reached statistical significance ($p=0.192$ and $p=0.026$, respectively).

![Graphs](image-url)

**Fig. 3.2** Individual relationships between post-race serum concentrations of adrenaline, noradrenaline and eHsp72 with post-race IL-6 serum concentrations for CSCI (O) and NON-CSCI (+). * Variable significantly explains variance in post-race IL-6 serum levels ($p<0.05$).
Table 3.2 Regression analyses with autonomic function indices and the inflammatory response to the wheelchair half-marathon. Reported as $R^2$ (r)

<table>
<thead>
<tr>
<th>Predictor variable</th>
<th>IL-6 post-race</th>
<th>Adrenaline post-race</th>
<th>Noradrenaline post-race</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adrenaline post-race</td>
<td>44% (0.66)*</td>
<td>NA</td>
<td>67% (0.82)*</td>
</tr>
<tr>
<td>Noradrenaline post-race</td>
<td>8% (0.29)</td>
<td>67% (0.82)*</td>
<td>NA</td>
</tr>
<tr>
<td>LF/HF RRI</td>
<td>8% (0.28)</td>
<td>29% (0.54)*</td>
<td>11% (0.33)</td>
</tr>
<tr>
<td>LF SBP</td>
<td>12% (0.35)</td>
<td>19% (0.44)</td>
<td>34% (0.58)*</td>
</tr>
<tr>
<td>TP SBP</td>
<td>29% (0.54)</td>
<td>33% (0.58)*</td>
<td>28% (0.53)</td>
</tr>
<tr>
<td>Adrenaline post-race</td>
<td>60% (0.77)*</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Noradrenaline post-race</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LF/HF RRI</td>
<td>14% (0.36)</td>
<td>33% (0.58)</td>
<td>34% (0.59)</td>
</tr>
<tr>
<td>LF SBP</td>
<td>27% (0.52)</td>
<td>38% (0.61)</td>
<td>25% (0.50)</td>
</tr>
<tr>
<td>TP SBP</td>
<td>81% (0.90)*</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Adrenaline post-race</td>
<td>88% (0.94)*</td>
<td>N/A</td>
<td>N/A</td>
</tr>
</tbody>
</table>

Abbreviations: IL-6 = interleukin-6; LF/HF RRI = the ratio of the power in low and high frequency of the heart rate variability; LF SBP = the power in the low frequency of systolic blood pressure variability; TP SBP = total power of systolic blood pressure variability.
* Regression model significantly explains variance in dependent variable ($p<0.05$)

3.5 Discussion

This study showed that the acute IL-6 response to a wheelchair half-marathon is attenuated in CSCI compared to NON-CSCI athletes, predominantly associated with the blunted catecholamine response observed in this group. Although autonomic function indices assessed at rest do not seem to be strong independent predictors for the IL-6 response to exercise, when used in combination with catecholamines they enhanced the predictive value of autonomic function assessments. This suggests that the influence of autonomic dysfunction on the dampened IL-6 response in people with CSCI is mediated by more than catecholamines only. Finally, eHsp72 was not elevated after the wheelchair half-marathon, suggesting that more intense or longer duration upper-body exercise is needed to increase its release into the circulation.
The autonomic function indices at rest (i.e. LF/HF RRI, LF SBP and TP SBP) differed between CSCI and NON-CSCI, indicating a difference in autonomic function between the 2 groups. Importantly, Claydon and Krassioukov (2008) observed a strong correlation between those measures and clinical outcomes of autonomic dysfunction (e.g. the catecholamine, HR and blood pressure response to the tilt manoeuvre as well as the sympathetic skin responses). As suggested by these authors, this may provide practitioners with an easy-to-use and non-invasive tool to assess autonomic function in people with SCI (Claydon & Krassioukov, 2008). Further indicating a difference in autonomic function between NON-CSCI and CSCI, the latter showed lower plasma concentrations of adrenaline and noradrenaline at rest and in response to exercise.

Previous research using only catecholamines as an indication of autonomic function has highlighted the importance of an intact sympathetic nervous system for the elevation of IL-6 following exercise (Ogawa et al., 2014; Paulson et al., 2013). Indeed, in the current study catecholamines explained 60% of the variance in serum IL-6 levels post-race. Studies infusing adrenaline in persons at rest support the notion that adrenaline can independently elevate circulating IL-6 concentrations (Sondergaard et al. 2000; Steensberg et al. 2001). Animal studies using adrenergic receptor antagonists suggest that adrenaline stimulates IL-6 production by the activation of β-receptors (De Rijk et al. 1994), which in turn leads to an increase in intracellular cAMP (Langfort et al., 2003). This can directly stimulate IL-6 production (Zhang et al., 1988). Additionally, the impact of adrenaline on muscle glycogen breakdown may mediate its stimulating effect on IL-6 production (Jensen et al., 1999). Indeed, exercise in a glycogen depleted state leads to an exacerbated acute IL-6 response (Bishop et al., 2001). However, the infusion of similar adrenaline levels as observed during exercise results in a much lower elevation in IL-6 when compared to exercise (Steensberg et al., 2001), suggesting that exercise provides additional stressors that cause the elevation of circulating IL-6 concentrations.

As circulating catecholamines seem to only partly explain the acute IL-6 response to exercise (Steensberg et al. 2001), other consequences of autonomic dysfunction such as the altered vascular tone and sympathetic innervation of the heart might further impact on the capacity to induce an
inflammatory response through exercise (Paulson et al., 2013). Autonomic function indices taken at rest are associated with clinical symptoms of autonomic dysfunction (Claydon & Krassioukov 2008), but also exercise performance (West et al., 2015). When used in isolation, the predictive value of supine LF/HF RRI, LF SBP and TP SBP in the present study seem to be limited with regards to the inflammatory response. However, the addition of these measures to the regression model with catecholamines significantly enhanced the explained variance of post-race serum IL-6 concentrations. This suggests that a combination of exercise-induced (catecholamines) and resting autonomic function indices (supine LF/HF RRI, LF SBP and TP SBP) can provide additional insight into the role of sympathetic dysfunction in the acute IL-6 response to exercise in people with SCI. This may be used to inform individual exercise prescription as well as the creation of additional strategies to promote health in people with SCI (Leicht et al., 2015). From a mechanistic perspective, the added predictive value of autonomic function indices at rest suggests that the impact of autonomic dysfunction on the dampened acute inflammatory response to exercise is indeed mediated by other factors than blunted circulating catecholamine concentrations only.

The close link between autonomic function and the catecholamine response to exercise makes it difficult to suggest what is accounted for by the resting autonomic function indices that is not accounted for by post-race catecholamine concentrations. In this respect it is noteworthy that athletes with autonomic complete CSCI might still experience autonomic reflexes below the lesion, resulting in spill-over of noradrenaline into the circulation (Leicht et al., 2013). Interestingly, one participant with CSCI showed a 4-fold and 2.5-fold increase in adrenaline and noradrenaline post-race respectively, despite being classified with a complete lesion and showing OH during the sit-up tilt test. Moreover, this was accompanied by an almost 4-fold increase in IL-6 following the race. Therefore, the impact of sympathetic reflexes and additional consequences of autonomic dysfunction other than lowered catecholamine concentrations on the acute inflammatory response to exercise would be an intriguing subject for future research.

While there is now sufficient evidence for the ability of upper-body exercise to induce an IL-6 response (e.g. Chapter 4, Kouda et al. 2012; Paulson et al. 2013), this is not yet the case for eHsp72.
In the current study no elevation of eHsp72 concentrations was detected in either CSCI or NON-CSCI. In a study investigating able-bodied participants, Leicht et al. (2016) reported an increase in eHsp72 after 45 min of arm-cranking at 60-65% peak power output in a non-permeable suit as opposed to the absence of an eHsp72 response after the same bout of exercise in conventional sport clothes. Since the exercise bout in the non-permeable suit resulted in increased heat storage, the results of this study suggest an important role of body temperature in the eHsp72 response. Since studies on lower-body exercise have shown increases in eHsp72 levels following relatively moderate bouts of exercise (Walsh et al., 2001), the reason for the attenuated eHsp72 response following upper-body exercise might partly lie in the limited muscle mass involved, inducing smaller increases in core temperature during exercise (Price, 2006). On the other hand, thermoregulation during exercise is impaired in individuals with SCI, possibly resulting in higher attained core temperatures (Price & Campbell, 2003). Future research in applied settings should therefore attempt to monitor core temperature during competition to shed more light on this measure as a potential mediator of the inflammatory response (Laing et al., 2008; Whitham et al., 2007).

The strong trend for elevated resting eHsp72 levels in CSCI compared to NON-CSCI is an interesting secondary finding of this study. Elevated resting levels of eHsp72 have recently been linked to insulin resistance and elevated resting levels of TNF-α, making it a possible marker for chronic low-grade inflammation (Krause et al., 2014). Since regular exercise can result in the downregulation of chronic low-grade inflammation (Petersen and Pedersen, 2005), it is tempting to suggest that the attenuated IL-6 response in the CSCI group, experienced over a long period of training which was not significantly different from the NON-CSCI group, may be implicated in the elevated basal levels of eHsp72.

In summary, the strong association between post-race serum IL-6 and catecholamine concentrations suggests a major role for the latter in the acute inflammatory response to a wheelchair half-marathon. While autonomic function indices assessed at rest were not predictive of the IL-6 response to the race when used in isolation, they enhanced the predictive value of autonomic function assessments when added to a predictive model with catecholamines alone. Therefore, the dampened
acute IL-6 response to a wheelchair half-marathon observed in people with CSCI may be influenced by more factors associated with autonomic dysfunction than solely blunted circulating catecholamine concentrations. Taking a wide range of factors associated with autonomic dysfunction into account may hence be of use to inform health promoting strategies to reduce chronic low-grade inflammation in individuals with CSCI.
Can intervals enhance the inflammatory response and enjoyment in upper-body exercise?

Sven P. Hoekstra, Nicolette C. Bishop, Christof A. Leicht
4.1 Abstract

Introduction As the acute inflammatory response to exercise can be dampened due to modality or disability-specific factors, alternative forms of exercise may help to reduce chronic low-grade inflammation. This study investigated the inflammatory and perceptual responses to 3 different forms of upper-body exercise, with a specific focus on high-intensity interval training (HIIT). Methods Twelve recreationally active, able-bodied males performed 3 external work-matched arm-crank sessions in a randomised order: 30 min moderate-intensity continuous (CON), 30 min moderate-intensity with changes in cadence (CAD) and 20 min HIIT. Blood samples were taken pre, post and 2 h post-exercise to determine plasma concentrations of interleukin (IL)-6 and IL-1ra. Perceptual responses pre, during and following the trials were assessed using the Feeling Scale, Felt Arousal Scale, rating of perceived exertion and the Physical Activity Enjoyment Scale. Results All trials induced an acute inflammatory response \((p<0.001)\), with similar increases in plasma IL-6 concentrations (fold increase; HIIT\(\geq1.25\pm0.20\), CAD\(\geq1.21\pm0.22\), CON\(\geq1.31\pm0.26\), \(p = 0.29\)) after exercise and in IL-1ra at 2 h post exercise (HIIT: 1.69\pm1.42, CAD: 1.27\pm0.25, CON: 1.38\pm0.43, \(p = 0.69\)) for all trials. More negative affect and higher ratings of perceived exertion were reported during HIIT compared to CON and CAD \((p = 0.016)\), whereas scores on the Physical Activity Enjoyment Scale reported after exercise were higher for HIIT (90.2\pm14.2) and CAD (92.4\pm8.7) compared with CON (78.8\pm10.2) \((p = 0.005)\). Conclusion When matched for external work, there was no difference in the inflammatory response to HIIT compared to moderate-intensity upper-body exercise. Although HIIT was (perceived as) more strenuous and affective responses were more negative during this mode, the higher Physical Activity Enjoyment Scale-scores for both HIIT and CAD reported after exercise suggest that the inclusion of variation enhances enjoyment in upper-body exercise. Therefore, as the fashion in which upper-body exercise is performed does not seem to influence the acute inflammatory response, it may be advised to prescribe varied exercise to enhance its enjoyment.
4.2 Introduction

It is widely recognised that regular exercise has protective effects against chronic low-grade inflammation associated chronic diseases, such as T2DM and CVD (Warburton et al., 2006). As is described in Chapter 1 (subheadings: “acute inflammatory response to exercise” and “chronic exercise to reduce chronic low-grade inflammation”), one of the proposed reasons is its anti-inflammatory effect (Petersen and Pedersen, 2005). The acute elevation of circulating IL-6 concentration and the resulting anti-inflammatory effect following exercise are dependent on both the intensity and duration of the session, with 126-fold increases in IL-6 seen after an ultra-running event (Nieman et al. 2005) and no increases seen after 30 minutes of moderate-intensity walking (Markovitch et al., 2008). Through an appreciation of the link between acute responses to exercise and its long-term effects, augmenting the acute inflammatory response to exercise may enhance its potential to combat chronic low-grade inflammation.

One increasingly popular form of endurance exercise is HIIT (Muller 1953). Whereas the more traditional continuous moderate-intensity exercise is recommended to last at least 30 min (Haskell et al., 2007), this less time consuming form of exercise consists of short bursts of high-intensity efforts of over 80% of VO2peak interspersed with low-intensity, active recovery (Gibala and Little 2010). Typically, HIIT sessions take not more than 20 min and have been shown to be equally or even more effective in inducing cardiovascular adaptations (Gibala and Little, 2012) and improving performance (Milanović et al., 2015) compared to moderate-intensity continuous exercise. Investigating the acute inflammatory response, Leggate et al. (2010) and Wadley et al. (2015) showed that the increase in IL-6 after HIIT was greater compared to moderate-intensity continuous exercise, although this finding has not consistently been shown in the literature (Cabral-Santos et al., 2015; Kaspar et al., 2016a).

While the aforementioned studies where all conducted in cycling or running, no such study exists for upper-body exercise. The smaller muscle mass involved in this form of exercise might hamper the acute inflammatory response (Bergfors et al., 2005; Hirose et al., 2004), making the
exploration of exercise modes that can augment this response even more relevant. Moreover, the relatively high prevalence of inactivity and chronic diseases in the population for which upper-body exercise is most suited (i.e. wheelchair users) further adds to this notion (Bauman and Spungen, 2008). As a recent review pointed out, alternatives to traditional moderate-intensity continuous exercise like HIIT might be a promising way forward to improve health in persons with a restricted ability to engage in lower-body exercise (Nightingale et al. 2017). Although bouts of moderate-intensity continuous upper-body exercise have previously been shown to be sufficient to provoke an acute inflammatory response (Paulson et al., 2015; Umemoto et al., 2011), this study will make a first step into the investigation of the acute inflammatory response to alternative forms of upper-body exercise (e.g. HIIT).

Despite the proposed health benefits of HIIT, these can only be achieved when engaging in this type of activity on a regular basis. The Hedonic theory states that people are more inclined to repeat behaviour that they find pleasant. Hence, it is suggested that the affective response to and enjoyment of exercise are important factors in exercise adherence (Ekkekakis et al., 2008b). Studies into the affective response during a bout of continuous exercise have shown a negative relationship between exercise intensity and affective responses, possibly making HIIT less suitable for health promotion. However, Bartlett et al. (2011) and Jung et al. (2014) showed that there might be a different mechanism involved in the perceptual responses to HIIT, shown by higher ratings of enjoyment (reported after the exercise bout) for HIIT compared with moderate-intensity continuous exercise despite more negative affective responses during HIIT. Whether this phenomenon also exists for upper-body HIIT is not yet clear. Possible factors that could alter the perceptual responses to upper-body exercise are the unfamiliarity of the participants to the task, the more dominant role of peripheral fatigue (Paulson et al., 2013) and the altered substrate metabolism compared to lower-body exercise (Sawka 1986). Nevertheless, an initial study in persons with SCI showed that also the enjoyment of upper-body exercise might be enhanced using HIIT (Astorino and Thum, 2016). The enhanced ratings of enjoyment may be related to the feelings of accomplishment when completing the
high-intensity efforts (Jung et al., 2014). It is, however, not known whether intervals *per se*, without a change in exercise intensity, influence perceptual responses to exercise.

To gain further knowledge in the health promoting potential of interval upper-body exercise, this study investigated the acute inflammatory and perceptual responses to 3 different modalities of upper-body exercise, with a particular focus on HIIT as a possible alternative form of exercise to prevent or combat chronic low-grade inflammation in people restricted to engage in lower-body exercise.

### 4.3 Methods

**Participants**

Twelve recreationally active, able-bodied males volunteered for this study. All participants were recruited from a student population. After being informed about the study procedure, they signed an informed consent form at the start of the first visit. The study was approved by the Loughborough University ethical advisory committee, in accordance with the Declaration of Helsinki.

**Study Design**

Participants visited the laboratory on 4 occasions. All exercise tests were performed on a Lode Angio arm-crank ergometer (Lode, Groningen, The Netherlands). In the first visit, participants reported their physical activity levels using a bespoke questionnaire and a graded incremental exercise test (GXT) to exhaustion was performed to assess peak exercise capacity and determine the lactate anaerobic threshold (LTan). The cytokine and perceptual responses to the following exercise modalities, which were matched for external work, were compared:

- Moderate-intensity continuous exercise (CON): 30 min arm-cranking at 80% of the PO at LTan, with a target cadence of 80 rpm.

- Moderate-intensity exercise with changes in cadence (CAD): 30 min arm-cranking at 80% of the PO at LTan, alternating high cadence (1min at 110 rpm) with low cadence (1min at 50 rpm).
High-intensity interval training (HIIT): 20 min arm-cranking, alternating hard (1 min at 200% PO at the LTan) and easy (1 min at 40% of the PO at LTan), with a cadence of 80 rpm.

**Graded exercise test**

Prior to the GXT, the height of the arm-crank and position of the chair were adjusted so that the arms never exceeded shoulder height and would never be fully stretched during rotation. Heart rate was continuously measured using radio telemetry (Polar PE4000, Kempele, Finland) and \( \dot{V}O_2 \) was determined using Douglas bags, which were analysed with a Servomex (Servomex 1440, Servomex Ltd, Crowborough, UK). For BLa determination, capillary blood was taken from the right earlobe and analysed using a Biosen C-line (EKF Industrie, Elektronik GmbH, Barleben, Germany).

After a 3 min warm-up, followed by 2 min rest, the GXT commenced at 5 W, followed by incremental stages of 15 W every 3 min. Cadence was held constant at 80 rpm. Oxygen uptake and BLa were assessed in the last minute of every incremental stage and final minute. The test was stopped at volitional exhaustion or when the participant could no longer maintain the requested cadence. The highest \( \dot{V}O_2 \) was taken as the peak value, whilst the highest 30 s rolling average HR was taken as HR peak. The LTan was determined using the Dmax method (Cheng et al., 1992).

**Main trials**

Prior to all trials, participants refrained from exercise, caffeine, alcohol, whilst they standardised their diet in the 24 hours before the trials using a food diary. All trials commenced between 11am and 1pm after a 2-hour fast, with the specific starting times standardised within participants. The trials were performed in a randomised order, with at least 2 days between them. The exercise commenced with a 2 min warm-up at 20 W, immediately followed by the main trial. During the trials, HR was measured continuously and expired air was collected for 1 min during minutes 3, 11, 19 and 27 (latter only for CON and CAD) for \( \dot{V}O_2 \) determination. Participants were allowed to drink water *ad libitum.*
Blood analyses

Blood from an antecubital vein was drawn into a K3EDTA vacutainer pre-, directly post and 2 hours after completion of the trial. Prior to the pre- and 2 h post-exercise sample, participants were seated for 10 min. Directly after collection, plasma was separated using a centrifuge spun at 1500 g for 5 min and stored at -80°C until analysis. Haematocrit and Hb concentrations were determined using a microlitre centrifuge and a spectrophotometer, respectively, to correct for changes in plasma volume from baseline according to the method postulated by Dill and Costill (1974).

Interleukin-6 and IL-1ra concentrations were determined using an ELISA, purchased from R&D systems (Minneapolis, US). Samples were analysed in duplicate, with a CV of 8.5% and 7.8% for IL-6 and IL-1ra, respectively.

Perceptual measures

The acute affective responses were reported using the FS (Hardy and Rejeski, 1989) and the Felt Arousal Scale (FAS) (Svebak and Murgatroyd, 1985). A resting value was given prior to exercise and responses during exercise were reported from the fifth and sixth minute with 6 min intervals, followed by directly post- and 20 min post-exercise. Furthermore, a FS score for the complete session was requested 20 min post-exercise (session-FS (sFS)). Local, central and overall RPE (Borg et al., 1987) were reported during exercise for the same time points as the affective response, while a sRPE was given 20 min post-exercise.

Participants filled-out the Physical Activity Enjoyment Scale (PACES) (Kendzierski & De Carlo 1991) and reported their enjoyment on a 20 cm Visual Analogue Scale (VAS) (Svensson, 2000) (“Enjoyment”) 20 min post-exercise. On the same scale, participants reported how enjoyable they would find it to engage in this form of exercise for 2 to 3 times a week in the coming month if they had to rely on their upper-body for exercise as result of an injury (“Expected enjoyment”).

After completion of all 3 main trials, the 20 min post-exercise enjoyment examination was extended with the following question: “Which of the 3 exercise modalities did you enjoy most? (“Preference”)”. Furthermore, participants rated their fondness for each of the modalities on a 1-9
Likert scale separately (“Fondness”). Lastly, participants were asked to write down their reasons for the reported “Preference”.

**Statistical analysis**

Participant characteristics and outcome measures are given in means and SDs. When the assumption of normality was violated, identified by the Shapiro-Wilk test, data were log transformed before analysis. This had to be done for the IL-6 data. A Greenhouse-Geisser correction was applied when the assumption of sphericity was violated, which was tested with Mauchley’s sphericity test. Scale data were analysed with parametric tests, in accordance with Bishop & Herron (2015) who support the robustness of F-tests with regards to ordinal data, in line with data analysis in similar studies (Bartlett et al., 2011; Jung et al., 2014). A one-way repeated measures ANOVA was performed for BLa at the end of the trial, HR, $\dot{V}O_2$ (average over the complete trial) and for the scores on the PACES, enjoyment, anticipated enjoyment, FS, sFS and sRPE. A 2-way repeated measures ANOVA was performed for the affective responses pre, post and 20 min post exercise. For the perceptual responses during the sessions (FS, FAS and RPE) a regression analysis curve was fitted with the data of every time point during exercise. The slope of the regression curve, the regression coefficient (rc), for every individual was used as a measure of progression in the perceptual responses during exercise and a one-way repeated measures ANOVA was used to test for differences in the rc between modalities. A Chi$^2$ test was used to test the distribution of “Preference” for goodness-of-fit. For all ANOVAs, post-hoc Bonferroni corrected tests were used for further inspection when statistical significance was reached. Statistical significance was set at $p<0.05$.

The 22nd version of the statistical software package SPSS (SPSS inc, Chicago, IL) was used for all analyses.

**4.4 Results**

Participant characteristics and the results of the GXT are shown in Table 4.1. All participants were able to complete the 3 main trials. Mean HR, $\dot{V}O_2$ and final BLa were higher in HIIT compared
to CAD and CON. Only $\dot{V}O_2$ differed between the CON and CAD condition, with higher values during CAD (Table 4.2).

Table 4.1 Participant descriptives (n=12) and main incremental exercise test results.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>22.5±3.3</td>
</tr>
<tr>
<td>Body mass (kg)</td>
<td>76.0±11.9</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.80±0.08</td>
</tr>
<tr>
<td>Physical activity level (hours/week)</td>
<td>4.33±2.04</td>
</tr>
<tr>
<td>$P_O_{peak}$ (W)</td>
<td>107±24</td>
</tr>
<tr>
<td>$\dot{V}O_2$ (L/min)</td>
<td>2.72±0.69</td>
</tr>
<tr>
<td>Relative $\dot{V}O_2$ peak (mL/kg/min)</td>
<td>35.5±4.51</td>
</tr>
<tr>
<td>HR peak (beats/min)</td>
<td>177±17</td>
</tr>
<tr>
<td>$P_O$ at LTan (W)</td>
<td>65±15</td>
</tr>
</tbody>
</table>

Abbreviations: $P_O$peak: peak power output; $\dot{V}O_2$peak: peak oxygen uptake; HRpeak: peak heart rate; $P_O$ at LTan: power output at the lactate anaerobic threshold.

Table 4.2 Physiological outcomes and the perceptual responses during and 20 min after the 3 trials.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>CON</th>
<th>CAD</th>
<th>HIIT</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR (bpm)</td>
<td>117±15</td>
<td>127±17</td>
<td>151±12*</td>
</tr>
<tr>
<td>BLa (mmol/L)</td>
<td>3.15±1.11</td>
<td>5.27±4.54</td>
<td>9.63±2.24*</td>
</tr>
<tr>
<td>$\dot{V}O_2$ (L/min)</td>
<td>1.43±0.25</td>
<td>1.76±0.36*</td>
<td>2.04±0.38*</td>
</tr>
<tr>
<td>$\dot{V}O_2$/ $\dot{V}O_2$peak (%)</td>
<td>53.5±7.3</td>
<td>65.6±9.6*</td>
<td>76.5±8.7*</td>
</tr>
<tr>
<td>rc FS</td>
<td>0.00±0.06</td>
<td>-0.02±0.03</td>
<td>-0.11±0.12*</td>
</tr>
<tr>
<td>rc FAS</td>
<td>0.01±0.03</td>
<td>0.01±0.02</td>
<td>0.05±0.07</td>
</tr>
<tr>
<td>rc RPE local</td>
<td>0.08±0.05</td>
<td>0.06±0.06</td>
<td>0.19±0.18*</td>
</tr>
<tr>
<td>rc RPE central</td>
<td>0.06±0.05</td>
<td>0.05±0.07</td>
<td>0.19±0.12*</td>
</tr>
<tr>
<td>rc RPE overall</td>
<td>0.06±0.05</td>
<td>0.05±0.05</td>
<td>0.18±0.14*</td>
</tr>
<tr>
<td>sRPE</td>
<td>10.8±1.9</td>
<td>11.3±1.8</td>
<td>14.8±2.3*</td>
</tr>
<tr>
<td>sFS</td>
<td>3.08±1.08</td>
<td>2.75±1.22</td>
<td>1.25±2.14*</td>
</tr>
<tr>
<td>PACES</td>
<td>78.8±10.2</td>
<td>92.4±8.7*</td>
<td>90.2±14.2*</td>
</tr>
<tr>
<td>Fondness</td>
<td>4.42±1.89</td>
<td>5.92±2.15</td>
<td>6.00±1.28</td>
</tr>
<tr>
<td>Enjoyment</td>
<td>12.1±3.1</td>
<td>11.7±4.3</td>
<td>14.2±2.3</td>
</tr>
<tr>
<td>Expected enjoyment Preference N=2</td>
<td>9.8±4.1</td>
<td>11.1±3.9</td>
<td>12.3±3.7</td>
</tr>
<tr>
<td>Preference N=4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preference N=6</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: HR: heart rate; BLa: final blood lactate concentration; $\dot{V}O_2$: oxygen uptake; rc: regression coefficient; FS: Feeling Scale; FAS: Felt Arousal Scale; RPE: ratings of perceived exertion; sRPE: session ratings of perceived exertion; sFS: session feeling scale; PACES: physical activity enjoyment scale.

* Significantly different from other trial(s),  + significantly different from CAD and CON
Both IL-6 and IL-1ra plasma concentrations increased in response to the exercise bouts (Fig. 4.1). Plasma IL-6 concentrations were higher compared with the preceding time point immediately post as well as 2 h post-exercise ($p<0.001$), while levels of IL-1ra only increased at 2 h post-exercise ($p = 0.003$). No differences between the modalities or an interaction effect of “Time” x “Mode” was found for either of the cytokines (IL-6: $p = 0.19$; $p = 0.29$; IL-1ra: $p = 0.35$, $p = 0.69$).

![Graph of plasma IL-6 and IL-1ra concentrations](image)

Fig. 4.1 Plasma IL-6 and IL-1ra concentrations pre, post and 2 h after the 3 upper-body exercise modalities. CON: moderate-intensity continuous exercise, CAD: moderate-intensity change in cadence, HIIT: high-intensity interval training. Lines represent individual participants, while bars represent group mean. *significant difference compared to previous time points
The scores on the FS showed an effect for “Time” ($p = 0.03$), “Mode” ($p = 0.003$) as well as a Time x Mode interaction ($p = 0.016$). Scores on the FS in response to HIIT were lower post and 20 min post-exercise compared to the other 2 modalities. The FAS scores were increased directly post-exercise, without any differences between modalities (“Time” $p < 0.001$) (Fig. 4.2). The progression of the affective responses during exercise, as assessed by the rc FS and the rc FAS, demonstrated a sharper decrease in scores on the FS during HIIT compared to the other modalities ($p = 0.003$, Table 4.2), indicating more negative affect during this exercise mode. There were no differences in the rc FAS between the modalities ($p = 0.098$). Of the 3 differentiated RPE scores reported during exercise, RPE C and RPE O showed a stronger increase during HIIT compared with CON and CAD ($p = 0.004$; $p = 0.02$, respectively), reflecting the demanding nature of the HIIT protocol. Detailed kinetics of the perceptual responses during the trials are shown in Figure 4.2.

![Perceptual responses](image)

Fig. 4.2 Perceptual responses pre, during and after the 3 different exercise bouts. FS: Feeling Scale, FAS: Felt Arousal Scale, RPE L: local ratings of perceived exertion. * Denotes a significant difference between HIIT and the other modalities at that time point.
Although the sFS was lower for HIIT compared to CON and CAD \( (p = 0.002) \) and the sRPE after HIIT was higher than after both other modalities \( (p < 0.001) \), participants rated both CAD and HIIT as more enjoyable than CON, indicated by higher scores on the PACES \( (p = 0.005) \). In addition, a trend towards higher ratings of enjoyment for HIIT was reported with the VAS \( (p = 0.082) \). Although a similar trend was seen for fondness and expected enjoyment, neither of these 2 variables differed between modalities \( (\text{fondness: } p = 0.098; \text{expected enjoyment: } p = 0.101) \). Although more participants reported to prefer HIIT and CAD compared to CON (Table 4.2), this did not reach statistical significance \( (p = 0.37) \).

### 4.5 Discussion

This study showed that upper-body HIIT and moderate-intensity exercise matched for external work increase plasma concentrations of IL-6 and IL-1ra to a similar extent. Despite HIIT being perceived as more strenuous and resulting in more negative affect during this modality, both HIIT and CAD were rated as more enjoyable than CON. Since enjoyment is suggested to be important in exercise adherence (Ekkekakis et al., 2008), including variation may be a promising strategy to promote exercise adherence and health in individuals restricted to engage in lower-body exercise.

*Cytokine response to upper-body exercise*

The increase of both plasma IL-6 and IL-1ra concentrations after CON, CAD and HIIT adds further support to the anti-inflammatory potential of upper-body exercise. While initially it was suggested that the limited muscle mass involved in arm-exercise might be insufficient to provoke an inflammatory response (Bergfors et al., 2005; Hirose et al., 2004a), recent studies have shown the elevation in plasma concentrations of IL-6 and IL-1ra after upper-body exercise ranging from 30 min at moderate intensity (Paulson et al., 2015) to a wheelchair marathon (Sasaki et al., 2014). Moreover, Leicht et al. (2016) recently showed a similar acute inflammatory response to arm- compared to leg-exercise when performed at the same relative intensity. Since the acute inflammatory response seems to be intensity and duration dependent, the initial lack of responses in IL-6 after upper-body exercise...
might have been caused by the short duration and the isolated muscle groups used for the exercise (Bergfors et al., 2005; Hirose et al., 2004). Indeed, as in running and cycling, the increases in IL-6 following upper-body exercise show a positive relationship with the physical demands of the exercise bout, with a ~1.8 fold increase found in the current study and an almost 20-fold increase after a wheelchair marathon (Sasaki et al., 2014).

In that light it might be conceived as somewhat surprising that the increase of IL-6 and IL-1ra did not differ between the 3 exercise modalities used in this study, despite higher RPEs during HIIT. However, Fischer (2006) highlights the importance of exercise duration in particular for the elevation of IL-6 plasma concentrations, which may be a reason why the shorter duration HIIT protocol did not induce a more pronounced inflammatory response when compared to the other modalities. Indeed, the present study suggests that the amount of “work” done is of greater importance than the fashion in which this work is prescribed. The execution of high-intensity bursts, with the accompanying rise in Bla, does not seem to have additional effects on the response of the cytokines measured in this study. This corroborates with studies examining the acute inflammatory response to different forms of cycling (Cabral-Santos et al., 2015), although larger increases in IL-6 after HIIT compared to work-matched moderate continuous exercise have been found as well (Leggate et al., 2010). Of note, as in the study of Leggate et al. (2010) the main trials of the current study were matched for external work rather than energy expenditure. This could be a possible limitation due to the possible influence of some aspects of the different trials on energy expenditure (e.g. the influence of cadence and intensity on arm-cranking mechanics and hence efficiency in individuals unaccustomed to upper-body exercise).

Perceptual responses to upper-body exercise

While a relatively large body of literature exists on affective responses and ratings of enjoyment to different forms of lower-body endurance exercise (Bartlett et al., 2011; Jung et al., 2014; Kilpatrick et al., 2015), this is not the case for exercise performed with the upper-extremities. Insight in this modality could especially be useful for disabled, elderly or obese individuals, for which this
form of exercise can be a suitable alternative to cycling or running. However, physiological as well as perceptual responses seen in cycling and running are not necessarily transferable to upper-body exercise. Differences in fibre type composition, substrate metabolism and the larger role for peripheral fatigue during upper-body exercise could alter the perceptual responses compared to leg exercise (Sawka 1986). Moreover, arm-cranking is a task that most able-bodied and recently injured individuals are unaccustomed to, in contrast to cycling or running. Nevertheless, in accordance with studies on lower-body exercise (Bartlett et al., 2011; Jung et al., 2014), in the current study higher ratings of enjoyment were reported after completion of the intermittent exercise modes when compared to the continuous modality. Interestingly, this was despite higher RPEs and more negative affective responses during HIIT. This seemingly contradictory phenomenon in intermittent exercise may be an important finding for future research into the feasibility of HIIT to promote health in non-athletic populations.

The Dual-Mode theory suggests that the affective response during exercise is intensity dependent, with variability between individuals in affective responses at intensities around the LTan, but an almost anonymous decline in affect during exercise intensities that surpass the LTan (Ekkekakis et al., 2008b). For this reason, the intensity of the moderate intensity trials in the current study was set below this threshold. Our results support the Dual-Mode theory, with lowest scores on the FS during HIIT, the only modality where participants surpassed the LTan. In a previous study using bouts of continuous lower-body exercise, the negative affective responses during exercise were also reflected in the ratings of enjoyment after exercise (Jung et al., 2014). However, the link between affective responses during exercise and post-exercise ratings of enjoyment might be different for intermittent exercise, shown by the current and other studies (Bartlett et al., 2011; Jung et al., 2014; Kilpatrick et al., 2015). For example, participants in the current study mentioned “a feeling of accomplishment” and “less boring/more interesting” as reasons why they reported to prefer HIIT over the other modalities (data not shown). This discrepancy between affect and enjoyment could be further explained by the difference in construct that the 2 measures embody. While the affective response is a hedonic, core response based on direct bodily sensations, enjoyment is regarded as an
emotion, which is likely to require cognitive appraisal in addition. Ratings of enjoyment, therefore, might take into account factors such as the relevance of the session to achieve goals and the perceived ability of the participant to reach those goals (Martinez et al., 2015).

Nevertheless, the challenging character of HIIT is an often heard concern with regards to exercise prescription in non-athletic populations (Biddle and Batterham, 2015). However, studies in individuals with T2DM (Maillard et al., 2016) and patients with heart disease (Currie et al., 2013) show promise for the prescription of HIIT with carefully chosen intensities to incorporate into regular exercise routines. In both long-term training studies, adherence rates did not differ between HIIT and moderate-intensity continuous exercise (Currie et al., 2013; Maillard et al., 2016). In addition, a recent study showed that HIIT is tolerated and perceived more enjoyable than continuous arm-crank exercise in a group of individuals with SCI (Astorino and Thum, 2016). Together with the results of the current study, this suggests that also when performed with the upper body, intermittent and more challenging, but time efficient forms of exercise can be perceived as more enjoyable than continuous exercise. A novel finding of this study is that intermittent exercise without increases in intensity (which could enhance the “feelings of accomplishment”) can also enhance ratings of enjoyment, as shown by higher enjoyment for CAD compared to CON. This could be useful for individuals that are physically not yet ready to include HIIT in their exercise programme or perceive the high intensities as aversive. Whether the phenomenon of higher ratings of enjoyment despite larger (feelings of) effort seen in HIIT can be used to better promote exercise cannot be concluded based on the data of the current (acute) study. Promising evidence includes data by Williams et al. (2008) who showed that positive affective responses during exercise can predict increased physical activity levels 6 and 12 months later. For ratings of enjoyment and the comparison of different exercise modes, no prospective data on physical activity behaviour exists, neither for lower- nor upper-body exercise. In addition, future research on the health promoting potential of different modes of upper-body exercise should aim to include members of populations that can benefit most from upper-body exercise (e.g. wheelchair users), as it is not known how well the results of this study translate to these individuals.
Taken together, this study showed that 3 work-matched arm-crank modalities lasting 20-30 min can all induce an acute cytokine response. This response did not differ between CON, CAD and HIIT, suggesting that, if performed regularly, each modality could be equally effective in reducing chronic-low grade inflammation. This allows to focus on factors that could enhance exercise adherence rates, notably the perceptual responses. While HIIT was (perceived as) more strenuous compared with CAD and CON, both intermittent modalities were rated as more enjoyable, suggesting that the inclusion of variation *per se* can enhance the enjoyment of exercise. Whether the enhanced ratings of enjoyment indeed translate to higher adherence rates should be subject of future research.
The acute inflammatory response to hot water immersion in sedentary, overweight adults

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This chapter is part of a manuscript that is published in the Journal of Applied Physiology under the following title: *The acute and chronic effects of hot water immersion on inflammation and metabolism in sedentary, overweight adults.*
5.1 Abstract

Introduction Since body temperature mediates the acute inflammatory response following exercise, passive heating may be a viable tool to induce this beneficial response in people that are restricted to be physically active. This study investigated the efficacy of a single hot water immersion (HWI) session to induce an acute inflammatory response. Methods Ten sedentary, overweight (BMI: 31.0±4.2 kg/m²) males were immersed in water set at 39°C for 1 h (HWI_{pre}) and completed 1 h of seated rest at ambient temperature as a control condition (AMB). Venous blood was obtained prior to, immediately post and 2 h post-session for assessment of intracellular heat shock protein 72 in monocytes (iHsp72), the monocyte subset distribution and plasma concentrations of interleukin (IL)-6 and nitrite. Following a 2-week intervention period, consisting of 10 HWI sessions, the acute inflammatory response to HWI was re-assessed (HWI_{post}) using the same procedures as applied during HWI_{pre}. Results Plasma IL-6 (fold increase HWI_{pre}: 2.02±0.90) and nitrite concentration (fold increase HWI_{pre}: 1.52±0.40) as well as the proportion of intermediate and non-classical monocytes were higher immediately after HWI_{pre} compared with AMB (p<0.008). In contrast, the HWI protocol employed in the present study was not sufficient to increase iHsp72 expression (p = 0.57). There was no difference in the acute elevation of any of the inflammatory markers between HWI_{pre} and HWI_{post} (p>0.169). Conclusion A single HWI session induces an acute inflammatory response. This was, however, not accompanied by an elevated expression of iHsp72. Together, these findings provide rationale to investigate the potential of a chronic HWI intervention to reduce chronic low-grade inflammation and improve metabolic health.
5.2 Introduction

Chronic low-grade inflammation is as an important risk factor for several chronic diseases, such as T2DM and CVD (Ridker, 2003). The potential of exercise to induce an acute inflammatory response has been described in Chapter 1 (subheading: “acute inflammatory response to exercise”). Of particular interest in the context of HWI is the acute increase in Hsp72 (Krause et al., 2015). Functioning as a cell chaperone when found in the cytoplasm (i.e. iHsp72) and a danger signal when present in plasma (i.e. eHsp72), Hsp72 plays a major role in the maintenance of homeostasis and the inflammatory response to a range of stressors (Noble et al., 2008). Moreover, as a consequence of the divergent roles of intra- and extracellular Hsp72, Krause et al. (2015b) recently proposed to use the ratio of the 2 measures as a marker for chronic low-grade inflammation.

During exercise, core and muscle temperature increase; their amplitude dependent on the exercise mode, intensity and the environment in which the activity takes place (Gleeson, 1998). While many stressors related to exercise contribute to the inflammatory response (e.g. oxidative stress, hypoxia, glycogen depletion) (Morton et al., 2009), the increase in body temperature seems a particular strong stimulus, as evidenced by the enhanced IL-6, IL-1ra and Hsp72 response following exercise in hot when compared with thermoneutral or cold conditions (Cooper et al., 2010; Mestre-Alfaro et al., 2012; Rhind et al., 2001). Although it has been proposed that contracting muscle is the main driver of the inflammatory response following exercise (Febbraio and Pedersen, 2005), recent animal studies suggest that hyperthermia can elicit this response independently of muscle contraction (Welc et al., 2012). As a result, passive heating methods such as HWI and sauna bathing may stimulate the production of markers associated with the beneficial effects of exercise (Francois and Thomas, 2016) and could potentially form an alternative health promoting strategy for individuals with a restricted ability to be physical active (e.g. wheelchair users or the elderly).

Although the available research is still sparse, preliminary evidence suggests that passive heating by HWI can indeed induce an acute inflammatory response in humans (Faulkner et al., 2017; Leicht et al., 2015; Oehler et al., 2001). In both individuals with CSCI and able-bodied individuals, 1...
h of HWI in water set 2°C above the resting oesophageal temperature resulted in an acute increase of plasma IL-6 concentrations, while HWI of 2 h in water set at 39.5°C resulted in a significant increase in iHsp72 expression in monocytes. Furthermore, in a direct comparison between HWI and exercise at a similar rate of metabolic heat production, Faulkner et al. (2017) showed that HWI elicits similar elevations in eHsp72 and somewhat smaller, albeit significant elevations in plasma IL-6 concentrations.

Besides its suggested impact on the inflammatory profile, recent evidence indicates that regular HWI can improve vascular function (Brunt et al., 2016). These improvements may be mediated by the increase in the bioavailability of NO. This vasodilator is produced by endothelial nitric oxide synthase (eNOS) in response to, among other factors, an increase in blood flow and temperature (Rees et al., 1989). Besides its role as a vasodilator, NO may also improve glucose metabolism by promoting glucose delivery to skeletal muscle (Baron et al., 1994) or directly enhancing peripheral insulin-independent glucose uptake (Roberts et al., 1997). The physiological stressors imposed by HWI and the reported adaptations following a chronic intervention (Brunt et al., 2016) suggest an increased production and the involvement of NO in adaptations following HWI, respectively. However, the acute effect of HWI on its bioavailability in humans has not been directly assessed yet. Therefore, considering its suggested importance in adaptations previously observed following chronic HWI interventions (Hooper, 1999; Brunt et al., 2016) this marker could help to gain insight in the mechanisms behind the potential benefits of HWI.

While understanding of the potential of HWI to reduce chronic low-grade inflammation will be key in promoting its implementation in practice, adherence rates may partly depend on the perceptual responses to this relatively novel intervention. Indeed, enjoyment and the affective response are important determinants of future engagement in exercise training (Biddle and Mutrie, 2007). During exercise, the discomfort associated with sustained work at high-intensities negatively impacts on the affective response (Ekkekakis et al., 2011). Although no physical exertion is involved in HWI, the imposed thermal strain may result in feelings of displeasure and discomfort, potentially
This study investigated the acute inflammatory response to a HWI session in sedentary, overweight males, and whether this acute response is affected by a 2-week HWI intervention period. It was hypothesised that a single HWI session results in the elevation of IL-6, iHsp72, eHsp72 and the percentage of intermediate and non-classical monocytes immediately post-immersion, followed by an increased percentage of classical monocytes and a sustained elevation of iHsp72 at 2 h post-immersion. This acute inflammatory response was expected to be blunted in response to the acute HWI session following the chronic intervention.

5.3 Methods

Participants

Participants were sedentary (<2 hours exercise/week), overweight (BMI >27), otherwise healthy males. Exclusion criteria were the usage of anti-inflammatory medication and contraindications to engage in HWI. The latter was assessed with a medical health questionnaire according to the American College for Sport and Exercise Medicine guidelines for exercise testing and prescription (ACSM, 2013), while engagement in structured exercise was reported using a questionnaire. Written informed consent was provided by the participants after being instructed about the study procedures.

Procedures

An outline of the procedures is given in Fig. 5.1. Participants visited the laboratory for a HWI (HWIpre) and control trial (AMB) in a counterbalanced order, with a minimum of 72 h between the visits. Participants refrained from exercise, alcohol and caffeine and standardised their diet using a food diary in the 24 hours prior to the visits. All visits started between 8-10 am, with the starting consistently applied for each individual to account for a possible circadian rhythm in any of the
outcome measures. After an overnight fast, nude body mass, height, hip and waist circumference were measured and skinfold thickness was assessed at 4 sites (biceps, triceps, subscapular and supra iliac) (Durnin and Womersley, 1973) for the estimation of body fat percentage.

Fig 5.1 An overview of the study procedures. AMB = resting at ambient temperature; HWI_pre = hot water immersion prior to the chronic intervention period; HWI_post = hot water immersion following the chronic intervention period. The order in which AMB and HWI_pre were performed was counterbalanced.

Thereafter, participants underwent 15 min of seated rest in an environmental chamber (27°C, 40% humidity) for baseline measurements (Gagge et al., 1967). Following the “pre” blood sample, participants entered the water tank for HWI_pre or remained seated for another hour in the same conditions for AMB. This control condition (instead of immersion in thermoneutral water) was chosen because this study was designed to evaluate the effects of HWI as a stand-alone health intervention rather than to investigate the effects of an increase in body temperature per se. Evidence suggests that the effects of hydrostatic pressure on inflammatory markers are negligible (Laing et al., 2007).

During HWI_pre, participants were immersed up to the neck for 1 hour in water set at 39°C. Participants sat in an upright position and were allowed to drink water ad libitum. During both HWI_pre and AMB, measurements were taken every 15 min. Blood pressure (Microlife BP3AC1-1, Cambridge, UK) was measured in duplicate at the level of the heart, while thermal sensation (TS),
thermal comfort (TC) (Gagge et al., 1967) and basic affect (Hardy and Rejeski, 1989) were reported. Expired air was collected for 3 min into Douglas bags for the determination of VO$_2$ using a Servomex 1440 gas analyser (Servomex Ltd, Crowborough, UK). Tympanic temperature was measured with a tympanic temperature probe (Squirrel, Grant Instruments, Shepreth, UK), using cotton wool to cover the external canal of the ear. Rectal temperature ($T_r$) was recorded every 5 min throughout the trials, using a rectal probe (YSI 400 series, Ohio, USA) that was inserted 10 cm beyond the anal sphincter. Heart rate (Polar RS400, Kempele, Finland) was continuously measured throughout.

Immediately on completion of the session, a “post” blood sample was taken and participants rested seated in the environmental chamber for 30 min. Thereafter, nude body mass was measured and a breakfast snack was provided (Sainsbury breakfast biscuits; 212 kcal, 5.8 g fat, 34.3 g carbohydrates, 4.0 g protein). The change in nude body mass and water consumed was used to estimate sweat loss. Participants were then allowed to rest and perform light work such as reading. Two hours after completion of the session, the “post 2 h” blood sample was taken following 15 min of seated rest.

Following the first 2 visits, participants enrolled in an intervention period consisting of 10 HWI sessions, all executed within 14 days. The first 5 sessions of this period lasted 45 minutes, while the last 5 lasted 60 minutes. More details on the measurements during the intervention period are provided in Chapter 6. Three days after completion of the last session of the intervention period, a second HWI trial (HWI$_{post}$) was conducted to study the effects of the intervention period on the acute inflammatory response to HWI. The procedures during this session were identical to HWI$_{pre}$.

**Biochemical analyses**

Blood was collected in K$_3$EDTA (plasma markers) and sodium heparin (flow cytometry) tubes. The K$_3$EDTA tubes were spun down immediately for 5 min at 1500 g and 4°C, and plasma was stored at -80°C until batch analysis. Flow cytometry was used to assess changes in iHsp72 in monocytes and the distribution in monocyte subsets. In addition, changes in the expression of iHsp72
in the respective monocyte subsets were assessed. Sixty µL of whole blood was incubated together with 5 µL of PerCP-conjugated cluster of differentiation (CD)14 and 2.5 µL of PE-conjugated CD16 antibodies in the dark at room temperature for 15 min. Thereafter, samples were lysed (750 µL; Facs lysing solution (BD Biosciences, San Diego, US), washed (1.5 mL phosphate buffered saline) and fixed using Leucoperm (60 µL; BD Biosciences). Following permeabilisation (60 µL; Leucoperm, BD Biosciences) samples were incubated with 4 µL of FITC-conjugated Hsp70 antibody or isotype control for 30 min. Finally, samples were washed and resuspended in phosphate buffered saline prior to running through the Flow Calibur (BD Biosciences). All antibodies except CD16 (BD Biosciences) were purchased from Miltenyi Biotech (Teterow, Germany). Cell Quest software (BD Biosciences) was used for the analysis, collecting 100,000 events per sample. Compensation of the flow cytometer prior to the study was performed manually using a resting whole blood sample of a male volunteer not participating in the study. Monocytes were selected based on positive CD14 expression, whereafter the percentage of monocyte subsets (CD14++CD16- classical monocytes, CD14++CD16+ intermediate monocytes and CD14+CD16++ non-classical monocytes) was determined using the trapezoid method (Zawada et al., 2015). The iHsp72 expression in monocytes was determined using the geometric mean fluorescence intensity (GMFI) following subtraction of the isotype control GMFI.

For the analyses of NO all glassware, utensils, and surfaces were rinsed with deionized water to remove residual NO intermediates prior to plasma nitrite concentration analysis. Plasma samples were introduced to a gas-tight purge vessel via 200 µL injections into the septum at the top of the vessel. The nitrite concentration of the undiluted (non-deproteinized) plasma was determined by its reduction to NO in the presence of glacial acetic acid and aqueous sodium iodide (4% w/v). The spectral emission of electronically excited nitrogen dioxide, derived from the reaction of NO with ozone, was detected by a thermoelectrically cooled, red-sensitive photomultiplier tube housed in a Sievers gas-phase chemiluminescence nitric oxide analyzer (Sievers NOA 280i, Analytix Ltd, Durham, UK). The nitrite concentration was determined by plotting signal (mV) area against a calibration plot of sodium nitrite standards.
Interleukin-6 (High-sensitivity, RnD systems, Abington, UK) and eHsp72 (Amp’d HSP70 high-sensitivity, Enzo life sciences, Farmingdale, US) were measured in plasma, in duplicate, using ELISAs. For the determination of eHsp72 concentrations, plasma samples were diluted 1:4 prior to running the ELISA. The intra-assay coefficients of variation were 7.0% and 6.2% for IL-6 and eHsp72, respectively. A whole blood count was obtained using a Yumizen H500 cell counter (Horiba Medical, Montpellier, France) for the determination of leukocyte subsets, Hct and Hb. The latter 2 were used to correct the post and post±2h plasma IL-6 and eHsp72 concentrations for changes in plasma volume (Dill and Costill, 1974).

Statistical analyses

All values are given as mean ± SD. Normality of the data was checked using the Shapiro-Wilk test and a log transformation was performed when non-normality was detected. Log transformation was performed on the eHsp72 data. Analysis of variance (ANOVA) with repeated measures where appropriate was used to detect differences in the acute responses between AMB and HWIpre, and HWIpre and HWIpost. For all analyses, a Bonferroni corrected post-hoc test was used for exploration of the differences at every time point when significance was detected. Due to a difference in baseline plasma nitrite concentrations between HWIpre and AMB, the fold changes from “pre” to “post” concentrations were compared between both conditions, using a paired T-test. Correlations between appropriate outcome measures were computed using Pearson’s $r$. $R$ was calculated as the fold change in the eHsp72/iHsp72 ratio (Krause et al., 2015a). The 23rd version of the statistical package SPSS (SPSS inc, Chicago, US) was used for all analyses and statistical significance was set at $p<0.05$.

5.4 Results

Participants

Eleven males were enrolled in the study between May 2016 and April 2017. One participant dropped-out during the intervention period due to complaints about headaches following the HWI
sessions. The personal characteristics of the remaining 10 participants, who completed all trials of the study, are given in Table 1.

Table 5.1 Personal characteristics of the participants (N = 10).

<table>
<thead>
<tr>
<th>Parameter</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>33.2±10.1</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>173.2±6.9</td>
</tr>
<tr>
<td>Body mass (kg)</td>
<td>92.1±9.2</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>31.0±4.2</td>
</tr>
<tr>
<td>Fat percentage (%)</td>
<td>25.1±3.5</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>96.9±4.7</td>
</tr>
<tr>
<td>Hip circumference (cm)</td>
<td>103.9±4.1</td>
</tr>
<tr>
<td>Structured exercise (min/week)</td>
<td>38±54</td>
</tr>
</tbody>
</table>

Abbreviations: BMI = body mass index

**Physiological and perceptual responses to HWI**

The physiological and perceptual responses during HWI\(_{pre}\) and AMB are given in Table 5.2. During HWI\(_{pre}\), \(T_e\) increased from 37.1±0.6°C to 38.7±0.4°C (Fig. 5.2). Oxygen uptake at the end of the session was increased twofold, while HR increased from 67±14 to 105±13 bpm. Alongside the physiological and thermal strain of the HWI session, basic affect decreased, while TS and TC increased (representing an increased sensation of heat and a decreased comfort associated with the heat, respectively) during HWI\(_{pre}\).
Table 5.2 Physiological responses to the hot water immersion and control trial.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>AMB Pre</th>
<th>AMB 60 min</th>
<th>HWI&lt;sub&gt;pre&lt;/sub&gt; Pre</th>
<th>HWI&lt;sub&gt;pre&lt;/sub&gt; 60 min</th>
<th>HWI&lt;sub&gt;post&lt;/sub&gt; Pre</th>
<th>HWI&lt;sub&gt;post&lt;/sub&gt; 60 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tre (°C)</td>
<td>36.9±0.5</td>
<td>36.6±0.5</td>
<td>37.1±0.6</td>
<td>38.7±0.4*</td>
<td>37.0±0.3</td>
<td>38.5±0.3*</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>69±17</td>
<td>64±15</td>
<td>67±14</td>
<td>105±13*</td>
<td>68±12</td>
<td>104±10*</td>
</tr>
<tr>
<td>VO&lt;sub&gt;2&lt;/sub&gt; (L/min)</td>
<td>0.20±0.04</td>
<td>0.23±0.04</td>
<td>0.21±0.04</td>
<td>0.42±0.10*</td>
<td>0.19±0.0</td>
<td>0.37±0.03*</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>127±10</td>
<td>123±12</td>
<td>126±13</td>
<td>138±15*</td>
<td>118±15^</td>
<td>126±13^</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>86±9</td>
<td>85±10</td>
<td>83±9</td>
<td>78±9</td>
<td>79±11</td>
<td>71±12^</td>
</tr>
<tr>
<td>Basic affect</td>
<td>0.9±1.4</td>
<td>0.7±1.3</td>
<td>1.3±2.0</td>
<td>-1.1±2.2*</td>
<td>1.1±1.9</td>
<td>-1.2±1.9*</td>
</tr>
<tr>
<td>TS</td>
<td>4.9±0.6</td>
<td>4.8±0.8</td>
<td>5.1±0.9</td>
<td>7.4±1.0*</td>
<td>4.7±0.5</td>
<td>6.7±0.8*</td>
</tr>
<tr>
<td>TC</td>
<td>0.0±0.0</td>
<td>0.0±0.5</td>
<td>0.2±0.4</td>
<td>2.2±1.0*</td>
<td>-0.1±0.3</td>
<td>2.3±1.3*</td>
</tr>
<tr>
<td>Sweat loss (L)</td>
<td>N/A</td>
<td>0.17±0.19</td>
<td>N/A</td>
<td>1.1±0.6*</td>
<td>N/A</td>
<td>1.7±0.6*</td>
</tr>
</tbody>
</table>

Abbreviations: AMB = control trial; HWI<sub>pre</sub> = hot water immersion session prior to HWI intervention period; HWI<sub>post</sub> = hot water immersion session following HWI intervention period; Tre = rectal temperature; HR = heart rate; VO<sub>2</sub> = oxygen uptake; SBP = systolic blood pressure; DBP = diastolic blood pressure; TS = thermal sensation; TC = thermal comfort

* significant difference with AMB; ^ Significant difference between HWI<sub>pre</sub> and HWI<sub>post</sub>

Fig. 5.2 Rectal temperature during and following AMB, HWI<sub>pre</sub> and HWI<sub>post</sub>. AMB = resting at ambient temperature; HWI<sub>pre</sub> = hot water immersion prior to the chronic intervention period; HWI<sub>post</sub> = hot water immersion following the chronic intervention period. * Significantly different from AMB.
Plasma concentrations of IL-6 were higher compared to AMB immediately following HWI_{pre}, \( (p<0.001) \), reflecting a 1.8-fold increase as a result of the HWI_{pre} session. However, this was not accompanied by a rise in either eHsp72 \( (p = 0.60) \) or iHsp72 in total monocytes \( (p = 0.57) \) directly post or 2 h post-HWI_{pre} (Fig 5.3). The same was true for the expression of iHsp72 in classical monocytes \( (p = 0.655) \), intermediate monocytes \( (p = 0.192) \) and non-classical monocytes \( (p = 0.574) \).

\( R \) did not differ between HWI_{pre} and AMB when iHsp72 were considered in total monocytes \( (p = 0.477; \text{pre-post}, \text{pre-post}+2h, p = 0.759) \), classical monocytes \( (p = 0.465; \text{pre-post}+2h, p = 0.793) \), intermediate monocytes \( (p = 0.419; \text{pre-post}+2h, p = 0.672) \) or non-classical monocytes \( (p = 0.978; \text{pre-post}+2h, p = 0.654) \).

The distribution of monocyte subsets changed directly after HWI_{pre}, with an increase of the intermediate \( (p = 0.004) \) and non-classical monocytes \( (p = 0.008) \) (Table 5.3). Lymphocyte numbers increased to a larger extend directly following HWI_{pre} compared to AMB \( (p = 0.003) \). Neutrophil and lymphocyte numbers increased to a larger extend following HWI_{pre} compared to AMB (neutrophils; \( p = 0.001 \), lymphocytes; \( p = 0.001 \)). There was no difference between HWI_{pre} and AMB in the acute elevation of total leukocyte and monocyte numbers (leukocytes; \( p = 0.163 \); monocytes; \( p = 0.558 \)). As can be seen in Fig. 5.3, the increase in plasma nitrite concentrations directly following HWI_{pre} was larger compared to AMB \( (p<0.001) \). Finally, there was no correlation between the peak T_{core} attained during HWI_{pre} and the acute change in iHsp72 expression \( (r = -0.11, p = 0.77) \), plasma IL-6 \( (r = 0.23, p = 0.55) \) or nitrite concentrations \( (r = 0.04, p = 0.91) \). In HWI_{pre}, there was no correlation between the peak T_{core} attained and the acute change in iHsp72 expression \( (r = -0.11, p = 0.77) \), plasma IL-6 \( (r = 0.23, p = 0.55) \) or nitrite concentrations \( (r = 0.04, p = 0.91) \).
Table 5.3 The distribution of monocyte subsets in whole blood following hot water immersion and the control trial at ambient temperature.

<table>
<thead>
<tr>
<th></th>
<th>Classical monocytes (%)</th>
<th>Intermediate monocytes (%)</th>
<th>Non-classical monocytes (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMB pre</td>
<td>94.6±2.3</td>
<td>1.3±0.4</td>
<td>2.6±0.7</td>
</tr>
<tr>
<td>AMB post</td>
<td>94.0±4.8</td>
<td>1.3±0.3</td>
<td>2.4±0.5</td>
</tr>
<tr>
<td>AMB p2h</td>
<td>94.2±1.9</td>
<td>1.4±0.6</td>
<td>2.6±1.1</td>
</tr>
<tr>
<td>HWI pre</td>
<td>94.50±4.0</td>
<td>1.3±0.6</td>
<td>2.2±0.9</td>
</tr>
<tr>
<td>HWI post</td>
<td>91.2±5.8</td>
<td>1.7±0.7*</td>
<td>3.4±1.9*</td>
</tr>
<tr>
<td>HWI p2h</td>
<td>94.4±2.7</td>
<td>1.3±0.4</td>
<td>2.0±0.4</td>
</tr>
<tr>
<td>HWI pre</td>
<td>94.2±1.2</td>
<td>1.5±0.6</td>
<td>3.1±1.0</td>
</tr>
<tr>
<td>HWI post</td>
<td>93.9±1.7</td>
<td>1.4±0.4^</td>
<td>3.5±0.4*</td>
</tr>
<tr>
<td>HWI p2h</td>
<td>94.7±1.5</td>
<td>1.2±0.5</td>
<td>2.7±1.3</td>
</tr>
</tbody>
</table>

Date are mean±SD. Abbreviations: AMB = control trial; HWIpre = hot water immersion session prior to HWI intervention period; HWIpost = hot water immersion session following HWI intervention period, p2h = 2 hours post hot water immersion

*Significant difference with AMB; ^ Significant difference between HWIpre and HWIpost

Adaptations to the intervention period

Resting T_r (p = 0.22) and HR (p = 0.82) did not change as a result of the HWI intervention period. However, systolic blood pressure was significantly lowered prior (p = 0.04) and stayed lower at the end of HWIpost (p = 0.004) when compared to HWIpre. Diastolic blood pressure was lowered to a significantly larger extent at the end of HWIpost when compared to HWIpre (p = 0.001). Thermal sensation at the end of HWIpost was significantly lower than at the end of HWIpre (p = 0.01) and sweat loss during HWI was increased from 1.1±0.6 to 1.7±0.6 L (p = 0.001).

The IL-6, eHsp72 and iHsp72 response did not differ significantly following HWIpost compared to HWIpre (IL-6: p = 0.786, eHsp72: p = 0.448, iHsp72: p = 0.710). The same was true for Hsp72 expression in classical (p = 0.220), intermediate (p = 0.169) and non-classical monocytes (p = 0.250). Only an attenuation of the acute increase in intermediate monocytes following HWI was present in HWIpost (p = 0.023), resulting in the absence of an increase in this subset following HWIpost (p = 0.06). There were no differences in the acute change between HWIpre and HWIpost for total leukocyte (p = 0.36), monocyte (p = 0.92), lymphocyte (p = 0.07) and neutrophil (p = 0.56) numbers.
Finally, the acute change in plasma nitrite concentrations was similar between HWI<sub>pre</sub> and HWI<sub>post</sub> ($p = 0.302$) (Fig. 5.3).

![Graphs showing plasma concentrations of IL-6, eHsp72, iHsp72, and nitrite](image.png)

Fig. 5.3 The acute IL-6, eHsp72, iHsp72 and nitrite response following hot water immersion and resting at ambient temperature. Lines represent individual participants, while bars represent group mean. * Significant Time x Trial interaction compared with AMB.

### 5.5 Discussion

This study showed that HWI induces an acute inflammatory response, indicated by the elevated plasma concentrations of IL-6 and proportion of intermediate and non-classical monocytes immediately following the session. In addition, plasma nitrite concentrations were increased following HWI. In contrast, the thermal stress induced by the protocol employed in the current study did not induce an acute increase in iHsp72 expression or eHsp72 concentration. Finally, the acute inflammatory response to HWI was not affected by 2 weeks of passive heat treatment.
The observation that 1 h of HWI in water set at 39°C induced a significant increase in plasma IL-6 concentrations corroborates with the notion that increases in body temperature can serve as an independent stressor to induce an acute inflammatory response. Previous studies employing 1 h of HWI have shown comparable increases in plasma IL-6 concentrations to the current study (Faulkner et al., 2017a; Leicht et al., 2015), while 2 h of HWI results in a more marked IL-6 response (Laing et al., 2007). Consistent with exercise studies (Fischer, 2006), this suggests that the IL-6 response to HWI is dose dependent. In line with this, a more intense HWI protocol than used in the present study (i.e. longer duration or warmer water) may be required to induce changes in iHsp72 or eHsp72.

Oehler et al. (2001) reported an acute increase in monocyte iHsp72 following HWI of 2 h in water set at 39.5°C (resulting in a peak Tcore of ~39.1°C), while a session of 1 h did not result in elevated iHsp72 expression in skeletal muscle (peak Tcore: ~39.0°C) (Morton et al., 2007). On the other hand, Faulkner et al. (2017) reported an acute increase in eHsp72 concentration following immersion up to the waistline for 1 h in water set at 40°C (Peak Tcore: ~1°C increase from rest). As the acute inflammatory response to HWI seems dose dependent, it is conceivable that there may exist a threshold in core or muscle temperature or time accrued above this threshold that needs to be reached in order to induce an iHsp72 response. Using exercise as a stressor, Gibson et al. (2016) suggested that at least ~27 min above a Tcore of 38.5°C is needed to induce the upregulation of Hsp72 mRNA. In the current study, participants’ Trec exceeded 38.5°C for ~15 min only. As the same group suggested that Tcore needs to be maintained above 38.6°C for 57 min to elevate the eHsp72 concentration, the required heat stress might need to be even higher to induce an acute response in this marker (Gibson et al., 2014).

Although the HWI protocol used in this study did not elevate iHsp72 expression, the acute increase in IL-6 concentrations indicates that in analogy to exercise, passive heating can also induce an acute inflammatory response, possibly leading to the circulating anti-inflammatory milieu postulated as one of the benefits of exercise (Petersen and Pedersen, 2005). While it is now widely acknowledged that contracting skeletal muscle is the main source of IL-6 during acute exercise (Fischer, 2006), it is not clear whether this is also the case for HWI. However, skeletal muscle is
suggested to secrete IL-6 in response to increases in local temperature (Welc et al., 2012). Suggested mechanisms are the influx of calcium via the opening of TRPV1 (Obi et al., 2016) and the activation of HSF-1, which can result in the production of both IL-6 and Hsp72 (Welc et al., 2013). HWI for 1 h in water set at 40°C leads indeed to a muscle temperature increase of ~2.5°C (Faulkner et al., 2017).

In addition, circulating monocytes are potent producers of cytokines and may be a source of IL-6 found in the circulation following HWI (Asea et al., 2000). The acute recruitment of intermediate and non-classical monocytes seen following HWI in this study could indeed have led to increased IL-6 secretion into the circulation as these subsets are known to release more IL-6 in response to an in-vitro stimulant such as LPS (Heine et al., 2012). However, since monocytes only represent a small percentage of leukocytes, it is not known what the impact of acute changes in circulating monocyte subsets on circulating cytokines is (Walsh et al., 2011). Nevertheless, since the proportion of relatively inflammatory monocytes (i.e. intermediate and non-classical monocytes) at rest are positively associated with the risk for a range of chronic diseases (Wong et al., 2012), the acute shift following HWI found in this study provides rationale for further research into the potential of HWI interventions to chronically alter the distribution of monocyte subsets in the circulation.

While the interest in HWI to reduce chronic low-grade inflammation is a relatively recent phenomenon, its potential to increase blood flow and enhance vascular function is more established (Drummond, 1993). Nonetheless, we show for the first time an acute increase in the bioavailability of the vasodilator NO in response to HWI in humans, possibly mediated by the enhanced activation of eNOS in response to the increase in shear stress and/or local temperature (Förstermann and Sessa, 2012). Since the acute increase in NO following HWI has the potential to aid tissue blood flow and is implicated in the translocation of GLUT4 to the plasma membrane of skeletal muscle cells during exercise (Roberts et al., 1997), HWI has the potential to facilitate glucose disposal in skeletal muscle and other tissues (Baron et al., 1994; Franks et al., 2005). In support, animal studies suggest GLUT4 translocation (Goto et al., 2015) and enhanced insulin sensitivity in skeletal muscle (Gupte et al., 2011) following an acute HWI session. Of note, in the current study the acute effects of HWI on
glucose disposal were not assessed and the implications of an acute increase in NO bioavailability on glucose disposal are therefore only speculative.

If passive heating is to be successfully introduced as a health promoting intervention in practice, it is important to assess perceptual responses to provide insight into its potential to influence adherence rates to the intervention (Ekkekakis et al., 2011). In the current study, the perceptual responses during 1 h of HWI of indicated feelings of discomfort similar to those reported during HIIT in chapter 4 and other studies (Jung et al., 2014; Kilpatrick et al., 2015). This implies that further increases in water temperature or session duration may result in an activity that is difficult to adhere to (Decker and Ekkekakis, 2017). Therefore, although more intense HWI sessions than the one used in the current study seem to be needed to induce an acute Hsp72 response, the practical application of HWI sessions such as the one applied in the study of Oehler et al. (i.e. 2 h at 39.5°C)(2001) in the general population is questionable. Moreover, the absence of more positive affective responses during HWIpost as compared to HWIpre suggests that no short-term improvements in the perceptual responses can be expected as a result of regular engagement in HWI. Therefore, future studies could test different HWI protocols in an attempt to optimise the balance between delivering a HWI stimulus that evokes the acute inflammatory response and metabolic benefits without eliciting negative affective responses that have the potential to limit adherence to the intervention.

Taken together, a single HWI session of 1 h in water set at 39°C induces acute elevations in plasma IL-6 and nitrite concentrations, as well as the percentage of intermediate and non-classical monocytes. However, the HWI protocol employed in this study was not sufficient to induce elevations in eHsp72 and iHsp72 in monocytes. A 2-week acclimation period did not affect the acute inflammatory response to a HWI session. Despite the absence of a heat shock response, the acute inflammatory response and increase in NO availability provide rationale to study the effectiveness of chronic HWI interventions to reduce chronic low-grade inflammation and improve metabolic health.
Chapter 6

The effect of a chronic hot water immersion intervention on inflammatory and metabolic markers in sedentary, overweight adults

Sven P. Hoekstra, Nicolette C. Bishop, Steve H. Faulkner, Stephen J. Bailey, Christof A. Leicht

This chapter is part of a manuscript that is published in the Journal of Applied Physiology under the following title: *The acute and chronic effects of hot water immersion on inflammation and metabolism in sedentary, overweight adults.*
6.1 Abstract

Introduction Chronic low-grade inflammation is an increasingly recognised risk factor for chronic diseases such as type 2 diabetes mellitus. While engaging in exercise training can improve the inflammatory profile and metabolic health, this may not be accessible for individuals restricted to be physically active. This study investigated the potential of an alternative strategy, namely hot water immersion (HWI), to reduce chronic low-grade inflammation and improve metabolic health. Methods Ten sedentary, overweight (BMI: 31.0±4.2 kg/m²) males underwent 10 HWI sessions in a 2-week period (INT). During the sessions, water was set at 39°C and participants were immersed up to their neck. A fasted, resting blood sample was taken prior to and 3 days following the intervention period. Eight sedentary males (BMI: 30.0±2.5 kg/m²), were included as control participants (CON). Control participants visited the laboratory for 2 fasted, resting blood samples at the same time interval as the 2 blood samples taken for INT. Main outcome measures were intracellular heat shock protein 72 (iHsp72), plasma interleukin (IL)-6 and extracellular heat shock protein 72 (eHsp72) concentrations, and fasting glucose and insulin concentrations. Results Fasting glucose and insulin were reduced following INT compared with CON (glucose INT; pre: 4.44±0.93 mmol/L, post: 3.74±0.72 mmol/L; p = 0.04; insulin INT; pre: 68.10±44.65 pmol/L, post: 51.7±27.3 pmol/L; p = 0.03) which was accompanied by a larger reduction in resting eHsp72 concentration (p = 0.03). However, resting iHsp72 expression (p = 0.59) and plasma IL-6 concentration (p = 0.87) were not changed following the intervention period. Conclusion The results of this study provide support for the use of HWI to improve aspects of the inflammatory profile and glucose metabolism in sedentary, overweight males, and may have implications for improving metabolic health in individuals restricted to be physically active.
6.2 Introduction

In the ancient Roman and Greek era it was already believed that engaging in regular hot bath-therapy induces a range of health benefits (Fagan, 2001), while Scandinavia, the Middle-East and Asia also have a long-lasting tradition of using passive heating as a leisure time activity (Karagulle et al. 2011). Despite this widespread popularity, limited scientific data are available on the protective effect of passive heating treatments against chronic diseases such as T2DM and CVD. Nevertheless, a recent cross-sectional study reported a reduced risk for all-cause mortality and CVD with an increase in exposure to sauna bathing (Laukkanen et al., 2015). Although these data are promising, the exact underlying mechanisms explaining the health promoting effects of regular engagement in passive heating are poorly understood (Laukkanen et al., 2018). However, a reduction in chronic low-grade inflammation may explain part of the positive effects found in observational studies. Indeed, Laukkanen and Laukkanen (2018) found a negative association between the frequency of sauna bathing and resting plasma CRP concentrations.

In line with acute exercise studies (Fischer, 2006), Chapter 5 showed that a single HWI session induces an acute elevation in plasma IL-6 and nitrite concentrations as well as a shift in monocyte subset distribution. Together with supporting evidence (Faulkner et al., 2017; Leicht et al., 2015), therefore, there is an increasing body of literature showing the potential of HWI to induce an acute inflammatory response. However, only few chronic intervention studies on the potential of HWI to improve metabolic health exist. Nonetheless, the reduction in fasting blood glucose concentrations in patients with T2DM (Hooper, 1999) and resting plasma IL-6 concentrations in patients with chronic heart failure (Oyama et al., 2013) are promising initial results. These studies, however, focussed on clinical populations, did not address the mechanistic link between inflammatory and metabolic markers and provided little detail on the acute (thermo-)physiological responses to HWI.

To date, several reviews have highlighted iHsp72 as a potential driver of the beneficial adaptations following HWI treatments (Hooper et al., 2014; Krause et al., 2015b). Animal studies have indeed provided compelling evidence for the potential of chronic HWI interventions to elevate
basal iHsp72 expression (Chung et al., 2008; Kavanagh et al., 2016; Silverstein et al., 2014).

Interestingly, the elevations in basal iHsp72 expression were associated with improved postprandial glucose metabolism and a reduction in fasting glucose in both mice and non-human primates (Chung et al., 2008; Kavanagh et al., 2016; Silverstein et al., 2014). In support for the significance of iHsp72 in insulin signalling, pharmacologically elevating iHsp72 expression also improves insulin sensitivity (Silverstein et al., 2014), while iHsp72 knock-out mice develop insulin resistance (Drew et al., 2014). It is, however, not known whether a HWI intervention can also elevate iHsp72 expression in humans. The smaller acute T\text{core} increases reported in human compared to animal studies (Faulkner et al., 2017a; Gupte et al., 2009) might make HWI less effective as a strategy to elevate resting iHsp72 expression and improve metabolic health in humans.

In addition to the impact of regular HWI on iHsp72 expression, its potential benefits for metabolic health may be mediated by other markers of systemic inflammation. For instance, basal levels of eHsp72 are reported to be higher in persons with diabetes and positively associated with levels of body fat (Rodrigues-Krause et al., 2012), insulin resistance and more established markers of chronic-low grade inflammation (i.e. TNF-\alpha) (Krause et al., 2014). As eHsp72 can stimulate circulating immune cells to produce pro-inflammatory cytokines (Johnson and Fleshner, 2006), a reduction in resting eHsp72 may be indicative of an improved inflammatory profile. Furthermore, intermediate and non-classical monocytes are potent producers of pro-inflammatory cytokines (Heine et al. 2012) and an elevated proportion of these subsets in the circulation is linked with CVD and T2DM (de Matos et al., 2016; Wrigley et al., 2011). While Chapter 5 showed that an acute HWI session induced a shift in the distribution of monocyte subsets, it is not known how chronic HWI impacts on this distribution at rest. Finally, chronic nitrate supplementation studies have provided intriguing evidence for the potential of NO to improve several aspects of health, notably blood pressure (Kapil et al., 2014). Considering the suggested association of this vasodilator with glucose metabolism, an elevated NO bioavailability at rest may yet be another mechanism by which HWI could exert its beneficial effects on metabolic health (Gheibi et al., 2018).
Taken together, animal data show promise for the use of HWI to improve metabolic health. However, the limited number of human studies available necessitates further research into this intervention before it may be implemented as a health promoting strategy among populations restricted to be physically active. Therefore, this study investigated the effects of a 2-week HWI intervention period on markers of chronic low-grade inflammation and glucose metabolism. It was hypothesised that the intervention period would increase resting expression of iHsp72, while reducing IL-6 and eHsp72 concentrations. In line with Hooper et al. (1999), this was expected to be accompanied with a reduction in fasting glucose and insulin concentrations.

6.3 Methods

Participants

The participants described in Chapter 5 formed the intervention group (INT) in the current study. In addition, a control group (CON) was included, for which the same inclusion criteria as for the intervention group applied.

Procedures

Participants in INT undertook a 2-week HWI-intervention. Prior to (i.e. pre) and 3 days following the chronic intervention (i.e. post) anthropometric measures and a resting blood sample were taken. In addition, a resting blood sample was obtained 7 days following the “post” visit. In the 24 h prior to each visit, participants refrained from exercise, alcohol, caffeine and standardised their diet using a food diary. Physical activity levels in the 7 days preceding each visit were reported by the participants using the International Physical Activity Questionnaire. Participants in CON visited the laboratory twice for a resting blood sample and anthropometric measures at the same time interval as the “pre” and “post” blood sample for INT. During the time between their 2 visits, the participants in CON did not engage in any HWI sessions or other heat acclimation practices and followed their normal daily routine, diet and physical activity pattern. Control participants were recruited alongside the participants in INT and were therefore included throughout the year rather than in one particular season.
On the day of the blood sample collection, participants visited the laboratory after an overnight fast. Body mass, height, waist and hip circumference were measured. In addition, skinfold thickness was assessed at 4 sites (biceps, triceps, iliac crest and subscapular) for the determination of body fat percentage (Durnin & Wormersley, 1974). Following a 15 min seated rest, blood pressure was measured at the left arm in duplicate using an automated blood pressure monitor (Omron Intellisense M7, UK). Thereafter, a blood sample was collected by venepuncture. All blood samples were drawn between 8-10 am with the exact time held the same for each individual to control for a circadian rhythm in any of the outcome measures. Of note, participants in INT visited the laboratory twice prior to the intervention period as part of the study reported in Chapter 5. The baseline blood sample of their first visit was used as “pre”.

**Hot water immersion intervention**

Following the first resting blood sample, participants underwent a HWI intervention consisting of 10 HWI sessions, all performed within 14 days. The first 5 sessions lasted 45 minutes, while the last 5 lasted 60 minutes. This procedure was chosen following pilot work, which suggested that people would need a gradual start of the intense 2-week HWI intervention period in order to avoid participant drop-out. In all sessions the temperature of the water was set at 39°C and participants were immersed up to their neck. During the sessions, HR, aural temperature, thermal sensation, thermal comfort and basic affect were assessed at rest and at the end of every 15 min during HWI (Table 6.1).

**Biochemical analyses**

Flow cytometric analyses for the determination of iHsp72 expression in monocytes and the monocyte subset distribution, blood sampling, haematology and the analyses of plasma IL-6, eHsp72 and nitrite concentrations were performed as described in Chapter 5. Additionally, a Biosen C-line (Biosen, Barleben, Germany) was used to determine blood glucose concentrations in whole blood (Nowotny et al., 2012). Insulin was measured in plasma, in duplicate, using an enzyme linked immunosorbent assay (CV: 2.5%) (Mercodia AB, Uppsala, Sweden).
Statistical analyses

All values are given as mean ± SD. Data were log-transformed or the Wilcoxon signed rank test was used when the assumption of normality was violated, which was checked using the Shapiro Wilk test. Personal characteristics at baseline of the intervention and control group were compared using independent T-tests. The difference in the effect of the intervention between INT and CON was assessed using a 2 (time) x 2 (group) repeated measures ANOVA. The maintenance of the effects of HWI at 7 d following the post blood sample was assessed by repeated measures ANOVA using 3 time points (pre, post, 7 days post) in INT only. When significance was reached, Bonferroni corrected post-hoc tests were used for exploration of the differences at every time point. Correlations between appropriate outcome measures were computed using Pearson’s $r$. The homeostasis model assessment for insulin resistance (HOMA-IR) was determined using fasting glucose and insulin concentrations $((\text{insulin x glucose})/22.5)$ (Matthews et al., 1985). The 23rd version of the statistical package SPSS (SPSS inc, Chicago, US) was used for all analyses and statistical significance was set at $p<0.05$.

6.4 Results

The physiological responses during the HWI sessions of the chronic intervention are provided in Table 6.1.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Pre session 1-5</th>
<th>End session 1-5</th>
<th>Pre session 6-10</th>
<th>End session 6-10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tympanic temperature (°C)</td>
<td>35.3±0.4</td>
<td>37.5±0.2</td>
<td>35.1±0.3</td>
<td>37.5±0.3</td>
</tr>
<tr>
<td>TS (1 to 9)</td>
<td>4.8±0.5</td>
<td>6.6±0.2</td>
<td>4.9±0.4</td>
<td>6.7±0.2</td>
</tr>
<tr>
<td>Basic affect (-5 to +5)</td>
<td>1.0±1.0</td>
<td>0.0±2.0</td>
<td>1.2±1.6</td>
<td>-0.7±1.8</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>67±13</td>
<td>105±2</td>
<td>68±14</td>
<td>105±3</td>
</tr>
</tbody>
</table>

Abbreviations: TS = thermal sensation; HR = heart rate. Data are means of 5 sessions during INT. End = measurement taken in the final 30 s of the session. Session 1-5 lasted 45 min, while session 6-10 lasted 60 min.

Anthropometric and physiological measures

The baseline characteristics of INT and CON are shown in Table 6.2. There were no differences in anthropometrics and physical activity levels between both groups at baseline. Following the intervention period, body mass was not changed in INT (92.1±9.2 kg to 92.3±9.5 kg, $p = 0.92$).
Both systolic (127±9 mmHg to 118±15 mmHg, \( p = 0.05 \)) and diastolic blood pressure (86±9 mmHg to 79±11 mmHg, \( p = 0.01 \)) were significantly lowered following the intervention period. Resting HR was not affected by the intervention (69±16 bpm to 72±14 bpm, \( p = 0.54 \)).

Table 6.2. Participant characteristics at baseline.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>INT (N=10)</th>
<th>CON (N=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>33.2±10.1</td>
<td>32.0±7.5</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>173.2±6.9</td>
<td>176.9±4.2</td>
</tr>
<tr>
<td>Body mass (kg)</td>
<td>92.1±9.2</td>
<td>93.9±9.4</td>
</tr>
<tr>
<td>BMI (kg/m(^2))</td>
<td>31.0±4.2</td>
<td>30.0±2.5</td>
</tr>
<tr>
<td>Body fat percentage (%)</td>
<td>25.1±3.5</td>
<td>25.7±2.3</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>96.9±4.7</td>
<td>100.1±8.4</td>
</tr>
<tr>
<td>Hip circumference (cm)</td>
<td>103.9±4.1</td>
<td>109.4±5.1</td>
</tr>
<tr>
<td>Structured exercise (min/week)</td>
<td>38±54</td>
<td>29±44</td>
</tr>
</tbody>
</table>

BMI: body mass index

Inflammatory and metabolic measures

The effect of the intervention period on resting IL-6, iHsp72 and eHsp72 levels are shown in Fig. 6.1. Resting levels of IL-6 and iHsp72 in total monocytes were not altered following the intervention period (IL-6; \( p = 0.87 \), iHsp72; \( p = 0.59 \)). The same was true for the expression of iHsp72 in the monocyte subsets (classical monocytes, \( p = 0.139 \); intermediate monocytes, \( p = 0.386 \); non-classical monocytes, \( p = 0.778 \)). Extracellular Hsp72 was significantly lowered in INT compared to CON (\( p = 0.033 \)). This resulted in a significant decrease in \( R \) in INT as compared to CON when iHsp72 was considered in total monocytes (\( p = 0.045 \)) and intermediate monocytes (\( p = 0.041 \)), but not in classical monocytes (\( p = 0.112 \)) and non-classical monocytes (\( p = 0.07 \)). The change in the distribution of monocytes subsets in the circulation was not significantly different in INT compared to CON following the intervention period (classical monocytes; \( p = 0.52 \), intermediate monocytes; \( p = 0.23 \), non-classical monocytes; \( p = 1.14 \) (Fig. 6.1).
Fasting blood glucose concentrations were significantly lowered in INT compared to CON following the intervention period \((p = 0.04)\) (Fig. 6.2). Fasting insulin concentrations did not change significantly in INT compared to CON \((p = 0.30)\). However, following inspection of the individual data an outlier was detected (Fig. 6.2, filled circles), which was confirmed using the methods for outlier detection postulated by Leys et al. (2013). After removing the insulin data of this participant, there was a significantly larger decrease in fasting insulin in INT compared to CON \((p = 0.04)\). HOMA-IR was also reduced to a significantly larger extent in INT compared to CON \((p = 0.03)\). Finally, there was no difference in the change of resting plasma nitrite concentrations between INT and CON \((\text{INT} 321\pm69 \text{ nM to 234}\pm64 \text{ nM}; \text{CON} 230\pm57 \text{ nM to 262}\pm77 \text{ nM}; p = 0.166)\).
Fig. 6.2 Fasting blood glucose and insulin concentrations before and after the intervention period for the intervention and control group. INT: intervention group, CON: control group. Lines represent individual responses, while bars represent the group mean. For fasting insulin, participant with filled circles was detected as an outlier and does not contribute to group mean. Significant effect for Time (\*) or Time x Group interaction (^)(p<0.05).

One week following the post blood sample, INT returned to the laboratory for a resting blood sample. Resting iHsp72 (pre: 307±53 GMFI, post: 309±69 GMFI, post+1 week: 358±116 GMFI; p = 0.22), IL-6 (pre: 1.22±0.52 pg/ml, post: 1.31±0.53 pg/ml, post+1 week: 1.12±0.65 pg/ml; p = 0.67), the percentage of classical monocytes (pre: 94.4±1.8%, post: 91.9±4.5%, post+1 week: 94.1±1.3%; p = 0.18), intermediate monocytes (pre: 1.25±0.38%, post: 1.69±0.73%, post+1 week: 1.47±0.51%; p = 0.27) and non-classical monocytes (pre: 2.70±0.92%, post: 3.10±1.09%, post+1 week: 3.39±1.35%; p = 0.28) were not changed compared to either pre or post. However, resting concentrations of eHsp72 were elevated compared to post (fold change pre-post: 0.83±0.41, fold change pre-post+1 week: 1.28±0.34, p = 0.01). The lowering of fasting blood glucose following the intervention period was still present at post+1 week (pre: 4.44±0.93 mmol/L; post: 3.74±0.72 mmol/L, post+1 week: 3.89±0.77 mmol/L, p = 0.001). However, fasting insulin was elevated at post+1 week compared to post (pre: 68.10±44.65 pmol/L, post: 51.7±27.3 pmol/L, post+1 week: 72.6±56.3 pmol/L, p = 0.05), returning to the insulin concentrations found prior to the intervention (pre- post+1 week, p = 0.21). As a result, there was a strong trend for an increase in HOMA-IR values at post+1 week compared to post (pre: 13.91±11.09, post: 8.99±7.89, post+1 week: 12.40±10.01, p = 0.06) and no difference in HOMA-IR
between post+1 week and pre \( (p = 0.47) \). Plasma nitrite concentrations were not changed at post+1 week compared to pre or post (pre: 314±61 nM, post: 247±66 nM, post+1 week: 304±91 nM; \( p = 0.09 \)).

**Correlations**

There was a negative correlation between plasma insulin concentration at baseline and its change following the intervention \( (r = -0.45, p = 0.01) \). There was no relationship with insulin at baseline and the change in blood glucose concentrations \( (r = 0.23, p = 0.33) \). No correlation was observed between baseline blood glucose concentration and the chronic change in insulin \( (r = -0.28, p = 0.27) \) or glucose concentrations \( (r = 0.29, p = 0.25) \). In addition, there was no correlation between the fold change in eHsp72 following the intervention and the change in insulin \( (r = 0.61, p = 0.06) \) or glucose concentrations \( (r = 0.03, p = 0.94) \). Finally, there was no correlation between the chronic change in iHsp72 expression and the chronic change in insulin \( (r = -0.16, p = 0.66) \) or glucose concentrations \( (r = 0.21, p = 0.56) \).

**6.5 Discussion**

In line with animal studies (Chung et al., 2008; Silverstein et al., 2014), the present study shows that also in humans HWI can exert beneficial effects on glucose metabolism. However, in contrast to the suggested importance of iHsp72, the reduction in fasting glucose and insulin concentrations occurred without a change in resting expression of this marker. The reduction in eHsp72 concentration at rest or the repeated acute inflammatory response observed following each HWI session may form part of an alternative explanation for the beneficial effects of HWI on metabolic health.

The findings of the current study corroborate with a pilot study conducted in individuals with T2DM in the late 1990s (Hooper et al. 1999). Fasting blood glucose concentration decreased significantly following a 3-week intervention period consisting of 16 HWI sessions of 30 min. Surprisingly, there is little additional human data to compare the results of the present study against.
Animal studies suggest increased basal iHsp72 levels as one of the potential reasons behind the beneficial changes in insulin sensitivity reported following HWI (Chung et al., 2008; Silverstein et al., 2014). Moreover, Hsp72 knock-out mice are highly insulin resistant and do not experience similar benefits from passive heating strategies compared to mice expressing Hsp72 (Drew et al., 2014). However, in the current study improvements in fasting glucose and insulin were found despite the absence of changes in iHsp72. One of the reasons for the discrepancy in the elevation of basal iHsp72 between the studies could be the limited rise in T\text{core} induced in the present study compared to the animal studies. For instance, Chung et al. (2008) increased the T\text{core} of mice up to 41.5°C, while rectal temperature in the current study reached 38.8°C only. Furthermore, in most animal studies iHsp72 was assessed in skeletal muscle (Chung et al., 2008; Gupte et al., 2009), while the current study assessed iHsp72 in monocytes. While the acute iHsp72 response to physiological stress (i.e. downhill running in the heat) in leukocytes follows a similar pattern as observed in skeletal muscle (Tuttle et al., 2017), it is not known whether the same is true for the expression at rest. For instance, while exercise training can elevate iHsp72 expression in skeletal muscle (Morton et al., 2009), marathon runners have a lower iHsp72 expression in monocytes compared with sedentary individuals (Fehrenbach et al., 2000). Of note, 6 days of repeated local heating of skeletal muscle increases its iHsp72 content at rest (Hafen et al., 2018). It may be that iHsp72 expression in monocytes may not be reflective of the main sites of glucose disposal (i.e. the liver and skeletal muscle) or that iHsp72 is not as crucial for insulin sensitivity in humans as previously suggested (Henstridge et al., 2014b; Krause et al., 2015b).

Despite no changes in resting iHsp72, eHsp72 concentrations were significantly lowered following the intervention period. When released into the circulation, eHsp72 can activate monocytes via the TLR4/CD14 complex, stimulating the secretion of pro-inflammatory cytokines such as IL-6, TNF-α and IL-β (Asea et al., 2002). As the latter cytokines can interfere with insulin sensitivity (Hotamisligil, 2003), it is suggested that eHsp72 exerts its deleterious effects on health via enhancing their release into the circulation (Johnson and Fleshner, 2006). Although the concept of eHsp72 as a danger signal for the innate immune system is relatively recent, cross-sectional data shows a positive
relationship between eHsp72 concentrations and both TNF-α and insulin resistance (Krause et al., 2014), but also vascular calcification (Krepuska et al., 2011) and the risk for acute coronary syndrome (Zhang et al., 2010). In contrast, however, resting eHsp72 concentration is lowered with increasing age (Njemini et al., 2011), which is also positively associated with chronic low-grade inflammation (Baylis et al., 2013). Additionally, the positive change in $R$ observed in INT might be indicative of an improved inflammatory profile (Krause et al., 2015a). Regarding the lowering of eHsp72 and $R$, however, the influence of both markers on glucose metabolism needs to be studied in more detail in order to appreciate the significance of both findings.

While previous studies have found changes in the inflammatory profile following short-term exercise and HWI interventions (Amorim et al., 2015; Hooper, 1999; Oyama et al., 2013), the relatively short duration of the HWI intervention period might have been the reason for the absence of changes in resting levels of iHsp72, IL-6, NO bioavailability and the monocyte subset distribution. The discrepancy between the present study and findings of other short-term intervention studies might be explained by the additional stressors associated with exercise (e.g. oxidative stress, hypoxia, glycogen depletion), the higher $T_{core}$ attained in most heat acclimation studies using exercise (Périard et al., 2016), or the more deteriorated health status at baseline of the participants in the chronic HWI studies (Hooper, 1999; Oyama et al., 2013). Furthermore, it is suggested that the initial beneficial effects of HWI might occur via the acute increase in NO availability following each bout of HWI, eventually leading to the elevation of basal NO and, consequently, iHsp72 expression (Malyshev et al. 2000; Krause et al. 2015b). A longer duration HWI intervention may therefore result in an increased resting expression of both markers. However, despite the lack of a change in most of the inflammatory markers assessed, it is striking that only 10 HWI sessions resulted in reductions in fasting glucose, insulin and blood pressure in males that were sedentary and overweight, but did not show signs of pre-diabetes or strongly elevated inflammatory markers at baseline. The latter may be a reason for the lack of changes in most of the resting inflammatory markers following the intervention. For instance, following a chronic exercise intervention, Lakka et al. (2005) observed a reduction in plasma CRP concentration in the group with an elevated concentration at baseline only. Indeed, the
positive correlation between baseline fasting insulin concentrations and the reduction in fasting insulin following the intervention suggests that those with more impaired metabolic health might benefit most from HWI.

The lowered blood pressure following the intervention period supports recent findings by Brunt et al. (2016), suggesting that HWI may also be a potent strategy to improve vascular health. While iHsp72- and NO-mediated mechanisms are suggested to play a role in this effect (Brunt et al., 2017), the improvements in blood pressure in the present study were independent of changes in resting levels of both measures. The acute increases in blood flow and shear stress during and following the HWI sessions might have improved vascular function independent of plasma nitrite concentrations at rest. Alternatively, it has recently been postulated that repeated acute salt losses induced by exercise may lead to reduced basal sodium concentrations in the circulation, stimulating vasodilation and reducing blood pressure (Turner and Avolio, 2016). Although an interesting hypothesis, this could not be directly tested with the data of the present study. Regrettably, resting blood pressure was not obtained in CON. Therefore, caution needs to be applied in the interpretation of the blood pressure findings in the present study.

The current study provides rationale to pursue further research on the potential of HWI to enhance (cardio)metabolic health. For instance, future studies should consider more robust measures of insulin sensitivity (e.g. oral glucose tolerance test or hyperinsulinemic-euglycemic clamp), implementing longer-term interventions and explore its effectiveness and feasibility in populations that may benefit most from this alternative health intervention (e.g. wheelchair users, frail elderly or those with other conditions that interfere with exercise participation). Moreover, including people with T2DM or central obesity may shed more light on the relevance of the observed reduction in fasting glucose and insulin for people experiencing more metabolic health problems. In addition, more intervention studies in humans are needed to clarify the role of inflammatory markers in glucose metabolism.
In summary, the 2-week HWI intervention period reduced fasting glucose and insulin concentrations, concomitant with lower resting eHsp72 concentrations. The reduction in fasting glucose and insulin, however, occurred independent of resting iHsp72 expression, plasma IL-6 and nitrite concentration, as these measures were not changed following the intervention. Together, this study provides support for the use of HWI to improve aspects of the inflammatory profile and glucose metabolism in sedentary, overweight males, and might have implications for improving metabolic health in populations unable to meet the current physical activity recommendations.
The effect of temperature and heat shock protein 72 on the ex-vivo acute inflammatory response in monocytes

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7.1 Abstract

Introduction The acute inflammatory response to active or passive activities that increase body temperature may aid to reduce chronic low-grade inflammation. This study investigated the impact of temperature and extracellular heat shock protein 72 (eHsp72) on the acute intracellular Hsp72 (iHsp72) and interleukin-6 (iIL-6) response in monocytes. Methods Whole blood was incubated for 2 h at 37.0°C, 38.5°C and 40.0°C, in the absence or presence of 0.5 µg/ml eHsp72. Flow cytometry was used to assess iHsp72 and iIL-6 expression in total monocytes and the 3 monocyte subsets. Results Incubation at 40.0°C (p<0.001) but not 38.5°C (p=0.085) increased iHsp72 expression in total monocytes when compared with 37°C (fold increase compared with 37°C; 38.5°C: 0.89±0.31, 40°C: 2.25±0.53), while there was no effect of temperature on iIL-6 expression (p=0.635). Following incubation with eHsp72, the expression of iHsp72 in classical monocytes was reduced at all temperatures (p<0.001), while there was a trend for a reduced iIL-6 expression (p = 0.071). Conclusion Large temperature elevations are needed to induce an acute inflammatory response in monocytes. In addition, contrary to its suggested role as a danger signal for the innate immune system, eHsp72 reduced iHsp72 expression in monocytes.
7.2 Introduction

The exercise-induced acute inflammatory response is suggested to partly mediate the reduction in chronic low-grade inflammation following chronic exercise training (Petersen and Pedersen, 2005). While this acute response following exercise is duration and intensity dependent (Fischer, 2006), the rise in body temperature is an additional determinant of its magnitude. Clamping of $T_{core}$ during exercise, for instance, dampens (Laing et al., 2007) or in some cases even completely abolishes the acute inflammatory response (Mestre-Alfaro et al., 2012; Rhind et al., 2004).

The acute inflammatory response to active (i.e. exercise) and passive body temperature elevations (e.g. HWI, sauna therapy) is characterised by the production of cytokines and other proteins, such as IL-6 and Hsp72. Although the increased expression of these inflammatory markers following physiological stress is manifested in a variety of tissues (e.g. skeletal muscle and adipose tissue), its assessment in monocytes provides a less invasive method to investigate such responses. Moreover, in comparison to other leukocyte subtypes, monocytes are particularly responsive to heat stress (Oehler et al., 2001). As monocytes are increasingly recognised for their influence on cardiovascular health (Heine et al., 2012), adaptations in this leukocyte subtype resulting from repeated heat exposure (e.g. increased basal iHsp72 expression) may aid to reduce chronic low-grade inflammation (Devaraj et al., 2008; Flynn and McFarlin, 2006; Henstridge et al., 2014).

Although monocytes have been shown responsive to heat stress (Oehler et al., 2001), the thermal stress needed to increase their expression of iHsp72 and IL-6 (iIL-6) is yet unknown. Immersion of humans in water set at 39.5°C for 2 h, for instance, induces an acute iHsp72 response (Oehler et al., 2001). However, a more modest thermal load (i.e. 1 h HWI in water set at 39°C) does not result in an increased iHsp72 expression (Morton et al., 2007; Chapter 5). While a temperature threshold may therefore exist for iHsp72, the effect of thermal stress on iIL-6 is less clear. Starkie et al. (2005) found no effect of additional heat stress during exercise on monocyte iIL-6 expression, and in-vitro incubation of monocytes at elevated temperatures can actually reduce iIL-6 production by macrophages (Fairchild et al., 2000). It should be noted that previous studies have assessed the acute
inflammatory response in total monocytes only (Oehler et al., 2001; Starkie et al., 2005). However, monocytes consist of 3 subsets (i.e. classical, intermediate and non-classical monocytes), differing in their expression and production of inflammatory markers in response to stress (Muckerjee et al., 2015). It is not known whether these subsets also differ in their acute response to temperature elevations.

Therefore, the aims of this study were twofold: to investigate 1) the influence of temperature elevations on the acute iIL-6 and iHsp72 response in monocytes and monocyte subsets, and 2) whether increased concentrations of eHsp72 mediate the acute inflammatory response in monocytes and monocyte subsets in response to elevated temperatures. Together, this can inform interventions that manipulate body temperature using passive (e.g. HWI, sauna therapy) or active methods (i.e. exercise) to induce changes in the inflammatory profile of monocytes.

### 7.3 Methods

**Procedures**

Twelve healthy, recreationally active males (age: 29.8±4.2 yrs; body mass index: 25.7±5.7 kg/m²; structured exercise: 4.0±4.2 hr/week) visited the laboratory following an overnight fast. Participants refrained from exercise, caffeine and alcohol on the day preceding the laboratory visit. After providing informed consent, participants rested in a seated position for 15 min. Thereafter, blood was drawn from an antecubital vein into a K₃EDTA tube. Six 1 ml aliquots of whole blood were pipetted into separate Eppendorf tubes. Whole blood was chosen to reduce the possible effects of separation techniques, while at the same time using a physiologically relevant model to study monocyte function (Mukherjee et al., 2015). The tubes were incubated for 2 h at 37.0°C, 38.5°C or 40.0°C using heat blocks, in the presence or absence of 0.5 µg/ml recombinant low endotoxin eHsp72 (Enzo life sciences, Farmingdale, US), 10 µg/ml Polymyxin-B (Enzo life sciences) and Brefeldin-A 5 µl/ml (Biolegend, San Diego, US) were added to all tubes. The temperature of each heat block was checked throughout using a calibrated thermometer, and the tubes were gently inverted every 30 min during the incubation period.
Flow cytometry

Immediately following incubation, 60 µl whole blood of each condition was added to 5 µl CD14, 2.5 µl CD16, 2 µl CD56 antibodies and 2 µl Fc-block (Miltenyi Biotec, Teterow, Germany). After 15 min incubation at room temperature in the dark, 750 µl lysing solution (BD Biosciences, San Diego, US) was added, and cells were incubated for another 10 min. Thereafter, cells were washed with 1.5 ml phosphate buffered saline (PBS). After fixation and permeabilisation of the cells using Leucoperm (BD Biosciences), 4 µl Hsp72 antibody, isotype control or 2 µl IL-6 antibody were added. After 30 min incubation at room temperature in the dark, cells were washed with PBS, resuspended in PBS and run through the flow cytometer (FACS Calibur, BD Biosciences). All antibodies except for IL-6 (Thermo Fisher Scientific, Rockford IL, US) were purchased from Miltenyi Biotec.

Cell Quest software (BD Biosciences) was used for the analysis of the 100,000 events collected for each condition. Following exclusion of CD56 positive natural killer cells, monocytes were selected based on positive CD14 expression (Ziegler-Heitbrock et al., 2010). The monocyte subset distribution (CD14++CD16- classical monocytes, CD14++CD16+ intermediate monocytes and CD14+CD16++ non-classical monocytes) was determined using the trapezoid method (Zawada et al., 2015). The iHsp72 and IL-6 expression in total monocytes and monocyte subsets were determined by subtracting the isotype control geometric mean fluorescence intensity (GMFI) from the GMFI of iHsp72 and iIL-6, respectively. See Fig S1 (supplementary material) for an illustration of the gating strategy used.

Statistical analyses

Outcomes are given in mean ± SD. Normality of the data was checked using the Shapiro Wilk test. A Greenhouse-Geisser correction was applied when the assumption of sphericity was violated, which was tested with Mauchley`s sphericity test. Repeated measures ANOVAs were conducted for all analyses, with a Bonferroni correction to test the effect of temperature and eHsp72 in the 4 related cell types (i.e. total, classical, intermediate, and non-classical monocytes). Significant main or interaction effects were followed up by Bonferroni adjusted post-hoc paired T-tests. Due to problems
with monocyte subset identification for 2 participants, \( N = 12 \) for total monocytes and \( N = 10 \) for the monocyte subsets. The 23rd version of SPSS (Chicago, USA) was used for all analyses.

### 7.4 Results

In the control condition (incubation at 37.0°C in the absence of eHsp72), iHsp72 expression in non-classical monocytes was lower when compared with intermediate and classical monocytes (\( p = 0.009 \)). Intracellular IL-6 expression was higher in intermediate and non-classical compared with classical monocytes (\( p = 0.018 \)) (Fig. 7.1). The monocyte subset distribution following the control condition was as follows: classical monocytes: 92.6±2.3%, intermediate monocytes: 1.28±0.88%, non-classical monocytes: 2.46±0.94%. The proportions of classical (\( p = 0.240 \)), intermediate (\( p = 0.174 \)) or non-classical monocytes (\( p = 0.232 \)) were not affected by temperature elevation, nor incubation with eHsp72 (classical monocytes \( p = 0.293 \); intermediate monocytes \( p = 0.176 \); non-classical monocytes \( p = 0.233 \)).

**The effect of temperature elevations on the acute inflammatory response**

Incubation at 40.0°C resulted in an increased iHsp72 expression in total (\( p<0.001 \)), classical (\( p<0.001 \)), intermediate (\( p<0.001 \)) and non-classical monocytes (\( p = 0.002 \)), compared with incubation at 37.0°C and 38.5°C (Fig. 7.1). There was a Temperature x Monocyte subset interaction, with lower iHsp72 expression in non-classical monocytes following incubation at 40.0°C compared with the other monocyte subsets (\( p<0.001 \)). There was no difference in iHsp72 expression for total monocytes or monocyte subsets between 37.0°C and 38.5°C (\( p>0.085 \)). There was no effect of temperature on the iIL-6 expression in total monocytes (\( p = 0.635 \)) or any of the monocyte subsets (\( p>0.412 \)).
Fig. 7.1 The expression of iHsp72 and IL-6 in total monocytes and monocyte subsets following 2 h incubation at 37.0℃, 38.5℃ and 40℃. Significantly different from (*) other temperatures and (^) other monocyte subsets ($p<0.013$). N = 12 for total monocytes; N = 10 for monocyte subsets.

The effect of eHsp72 on the acute inflammatory response

Incubation with eHsp72 resulted in a lower iHsp72 expression in total monocytes at all temperatures ($p<0.001$)(Fig. 7.2). The same effect was present in classical monocytes ($p<0.001$), but not in intermediate ($p = 0.436$) and non-classical monocytes ($p = 0.920$). There was a trend for a lower iIL-6 expression following incubation with eHsp72 ($p = 0.074$). This trend was also present in classical monocytes ($p = 0.074$), but not in intermediate ($p = 0.693$) or non-classical monocytes ($p = 0.260$).
Fig. 7.2 The effect of incubation with eHsp72 on hHsp72 and IL-6 expression in total monocytes and monocyte subsets at 37.0°C, 38.5°C and 40°C. White and black bars represent incubation in the absence and presence of eHsp72, respectively. * Significant effect of eHsp72, ^ significant Temperature x Condition effect (p<0.013). N = 12 for total monocytes; N = 10 for monocyte subsets.
The interaction between temperature elevation and eHsp72

There was a significant Temperature x Condition interaction for iHsp72 expression in intermediate monocytes, where the presence of eHsp72 resulted in a larger increase in iHsp72 expression between 38.5°C and 40.0°C when compared with incubation in the absence of eHsp72 ($p = 0.004$) (Fig. 7.2). No interaction effects were present for iHsp72 expression in total, classical or non-classical monocytes ($p > 0.117$). There was also no Temperature x Condition interaction for iIL-6 expression in total monocytes ($p = 0.646$) or any of the monocyte subsets ($p > 0.641$).

7.5 Discussion

As incubation of whole blood at 40.0°C but not 38.5°C elevates iHsp72 expression, the results of the present study indicate that large temperature elevations are needed to increase the expression of this intracellular chaperone in monocytes. In addition, despite being referred to as a “danger signal” (Johnson and Flesner, 2006), incubation of whole blood with eHsp72 resulted in a significant reduction in iHsp72 and a trend towards a reduction in iIL-6 expression. Finally, differential acute responses to heat as well as eHsp72 were observed among the 3 monocyte subsets, stressing the importance of distinguishing between the subsets when assessing acute responses to potential health promoting strategies.

The observation that large temperature elevations are needed to elevate iHsp72 expression are in line with previous exercise and passive heating studies. Morton et al. (2007) and Chapter 5 showed that HWI for 60 min, resulting in a peak $T_{core}$ of 38.9±0.2°C and 38.7±0.4°C, respectively, fails to increase iHsp72 expression. On the other hand, Oehler et al. (2001) observed a significant increase in iHsp72 expression in monocytes following 2 h incubation of whole blood at 39°C, suggesting that a temperature threshold close to 39.0°C to increase iHsp72 production may exist. Supporting this notion, Gibson et al. (2016) reported that a $T_{core}$ higher than 38.5°C needs to be maintained for at least 27 min to induce the transcription of Hsp72 mRNA in leukocytes following exercise. Ultimately, the need for such large $T_{core}$ increases may hinder the feasibility of interventions aimed to increase iHsp72 expression in monocytes. It is noteworthy, however, that iHsp72 expression in monocytes constitutes
only part of the inflammatory profile and response to stress, improvements in metabolic health markers at rest are reported in the absence of acute changes in its expression (Hafen et al., 2018).

The absence of an acute iIL-6 response following heat stress is in line with other in-vitro studies, in which phagocytic capacity (Roberts and Steigbigel, 1977) and iIL-6 production of monocytes (Fairchild et al., 2000) were not affected by elevated temperatures. Moreover, additional heat stress during exercise does not alter monocyte iIL-6 production in response to LPS stimulation (Starkie et al., 2005). Therefore, it is conceivable that the increased plasma IL-6 concentrations following in-vivo passive heating reported in previous studies (Faulkner et al., 2017; Laing et al., 2007; Leicht et al., 2015) originated from tissues or organs other than monocytes (e.g. skeletal muscle).

Incubation of whole blood with eHsp72 reduced the expression of iHsp72 and, although non-significantly, iIL-6 in classical monocytes at all 3 temperatures. This contrasts with the suggestion that eHsp72 may serve to amplify the inflammatory response to heat stress by acting through the same mechanisms as a damage-associated molecular pattern after its secretion into the circulation (Gupta et al., 2010). While LPS indeed amplifies iHsp72 production during heat stress (Gupta et al., 2010), it may be that eHsp72 does not activate immune cells in an LPS-like manner in all situations (Van Eden et al., 2012). Indeed, although incubation with eHsp72 can induce cytokine production in monocytes and macrophages (Asea et al., 2000; Campisi et al., 2003; Galdiero et al., 1997), support for a mere anti-inflammatory role of eHsp72 exists. For instance, Ferat-Osario et al. (2014) showed that eHsp72 can downregulate the acute inflammatory response to stimulation with TLR agonists in peripheral mononuclear blood cells, while Hulina et al. (2018) observed similar anti-inflammatory actions of eHsp72 when cells of a monocytic cell line were incubated with a TLR2 agonist. Of note, the reduction in iHsp72 expression following incubation with eHsp72 was not present in intermediate and non-classical monocytes. As TLR stimulation by eHsp72 is CD14 dependent (Asea et al., 2000), the lower CD14 expression on non-classical monocytes may partly explain the lack of responsiveness to eHsp72 in this monocyte subset (Cros et al., 2010).
In summary, this study showed that incubation of whole blood at 40.0°C but not 38.5°C elevates iHsp72 expression in monocytes. Furthermore, incubation with eHsp72 reduced iHsp72 and, although non-significantly, iIL-6 expression in monocytes; which is in contrast with its suggested role as danger signal for the innate immune system. The potential practical implications of these results are that strategies aiming to increase iHsp72 expression in monocytes by repeated passive or active heating may need to induce large elevations in body temperature to do so.
Chapter 8

General discussion
An overview of the main findings of this thesis is shown in Fig. 8.1. In Chapter 2, the acute inflammatory response following 90 min of interval arm-cranking was lower compared with exercise involving a larger muscle mass (i.e. cycling). Furthermore, the acute iHsp72 response to upper-body exercise was further attenuated in individuals adapted to this exercise modality. In the field-study described in Chapter 3, the autonomic dysfunction present in people with CSCI, resulting in a markedly dampened catecholamines response to exercise, was strongly associated with the attenuated acute IL-6 response following a wheelchair half-marathon in this group. Together, this suggests that for individuals that are upper-body trained and/or those with sympathetic dysfunction, upper-body exercise may be less effective in inducing an acute inflammatory response. Consequently, this may make exercise less effective to reduce chronic low-grade inflammation in these individuals. Chapter 4 showed that HIIT can be a time efficient form of exercise to induce an acute inflammatory response, although the inclusion of high intensity bouts does not provide an additional stimulus for the acute elevation in cytokine concentrations other than that it increases the external work performed per time unit. In support of HIIT as a potential additional part of prescribed exercise regimes to reduce chronic low-grade inflammation, this form of upper-body exercise was perceived as more enjoyable than moderate-intensity continuous exercise. Apart from upper-body exercise, additional interventions may be warranted in people restricted to engage in lower-body exercise. Using the concept that the elevation in Tcore can partly mediate the acute inflammatory response to exercise, the efficacy of a widely applicable passive heating strategy (i.e. HWI) to improve the inflammatory profile was investigated in Chapters 5 and 6. A single HWI session induced an acute increase in plasma IL-6 and nitrite concentrations, while a 2-week intervention period reduced fasting glucose, insulin and eHsp72 concentrations, as well as resting blood pressure. However, acute or chronic HWI was not effective in changing iHsp72 expression in monocytes. Indeed, the ex-vivo experiment presented in Chapter 7 confirmed that large temperature elevations are needed to acutely elevate iHsp72 expression in monocytes.
Factors influencing the acute inflammatory response to exercise

Chapter 2
90 min upper-body interval exercise induces a smaller acute inflammatory response compared with lower-body exercise, which is further attenuated by chronic training adaptations in the upper body.

Chapter 3
Wheelchair athletes with CSCI have a dampened acute IL-6 response following a wheelchair half-marathon compared with non-CSCI wheelchair athletes, which is strongly associated with autonomic dysfunction.

Inducing and amplifying the acute inflammatory response

Chapter 4
20 min of upper-body HIIT induces a similar acute inflammatory response compared with 30 min moderate intensity upper-body exercise, while intervals enhanced enjoyment of the exercise session.

Chapter 5
A single HWI session acutely elevates plasma IL-6 and nitrite concentrations, but not eHsp72 concentration or iHsp72 expression.

Chapter 7
Incubation of whole blood at 40.0°C but not 38.5°C elevates iHsp72 expression in monocytes compared with incubation at 37.0°C. eHsp72 reduces the expression of iHsp72 in monocytes.

Improving the inflammatory and metabolic profile at rest

Chapter 6
A 2-week chronic HWI intervention reduces fasting glucose, insulin and eHsp72 concentrations as well as blood pressure, but does not affect iHsp72 expression or plasma IL-6 and nitrite concentrations.

Fig. 8.1 Overview of the main findings of this thesis. Abbreviations: CSCI = cervical spinal cord injury; IL-6 = interleukin-6; eHsp72 = extracellular heat shock protein 72; iHsp72 = intracellular heat shock protein 72; HWI = hot water immersion; HIIT = high-intensity interval training
8.1 Factors influencing the acute inflammatory response to exercise

Upper-body exercise is characterised by the involvement of a small active muscle mass, resulting in a lower cardiovascular strain when performed at the same relative intensity as lower-body exercise (Sawka, 1986). Since contracting skeletal muscle is a major contributor to the increased concentrations of circulating cytokines following exercise, the small muscle mass involved may therefore attenuate the acute inflammatory response to this form of exercise. In Chapters 2 and 3, factors that may influence the potential of upper-body exercise to induce an acute inflammatory response have been investigated. The acute elevation of plasma IL-6 concentrations and iHsp72 expression following arm-cranking and cycling was studied in people chronically trained in either of the 2 modalities to investigate the impact of the active muscle mass and modality-specific training status, while the influence of SCI-specific factors was studied using a wheelchair half-marathon as the exercise model.

Active muscle mass and body temperature

While early studies on the acute inflammatory response to upper-body exercise failed to observe an acute elevation in inflammatory markers (Bergfors et al., 2005; Hirose et al., 2004), results of the present thesis support the effectiveness of upper-body exercise to induce this beneficial response. As little as 20 min of upper-body HIIT significantly elevated plasma IL-6 and IL-1ra concentrations (Chapter 4). The absence of an acute IL-6 response in Bergfors et al. (2005) and Hirose et al. (2004) was therefore likely a result of the low-volume exercise employed in these studies. In Hirose et al. (2004), for instance, only 6 sets of 5 eccentric contractions with the elbow flexors were performed. Fischer (2006) indicated the importance of exercise volume (duration x intensity) for the acute elevation of plasma IL-6 concentrations. Indeed, previous studies using larger volume upper-body exercise, such as 20 min (Kouda et al., 2012) and 30 min arm-cranking (Paulson et al., 2014), are in line with the results of the present thesis. Nonetheless, the small muscle mass engaged in upper-body exercise may result in a limited acute inflammatory response compared with lower-body exercise such as cycling. However, Leicht et al. (2016) showed that exercise using either modality results in a similar acute inflammatory response when performed at the same relative
intensity. Therefore, following moderate-intensity exercise of the same duration, relative intensity seems to be the main determinant of the acute inflammatory response, independent of the muscle mass involved in the activity (Leicht et al., 2016). This is partly supported by Chapter 2, in which a higher absolute exercise intensity, resulting from exercise at the same relative intensity, performed by the group for whom the exercise was familiar did not lead to a larger acute elevation in IL-6 or iHsp72. However, in people with a lower physical capacity than the participants in Chapter 2, the absence of an increase in Tcore and associated stressors may impair the acute production of inflammatory markers. Indeed, Tcore in Chapter 2 increased to a similar extent in the accustomed and unaccustomed groups in both cycling and arm-cranking. For instance, Paulson et al. (2014) found no acute increase in plasma IL-6 and IL-1ra concentrations after 30 min of handcycling at 60% VO2peak in people with a SCI \( \leq T6 \); in contrast to the acute increase in both markers after exercise of similar duration and relative intensity observed in Chapter 4. Although speculative, the discrepancy may have been the result of the lower absolute external work performed in Paulson et al. (2014). Moreover, although performed at 50% VO2peak, walking does not elevate circulating IL-6 concentrations in sedentary, middle-aged men, possibly due to the low absolute workload performed during this type of exercise (Markovitch et al., 2008). At the same time, it should be acknowledged that a similar acute IL-6 response following 3 h of knee-extension exercise at less than half of the PO is observed in older individuals compared with young adults (Pedersen et al., 2004).

When comparing upper- and lower body exercise, the results of this thesis suggest that when exercise is of long duration and high intensity, upper-body exercise may in fact result in smaller acute elevations in IL-6 and iHsp72. The discrepancy between moderate-intensity exercise (Leicht et al., 2016) and the interval protocol employed in the present thesis may be explained by the difference in their impact on Tcore during exercise. As Tcore can mediate the acute inflammatory response to exercise (Laing et al., 2007; Rhind et al., 2004), the exacerbated difference in Tcore between upper- and lower-body exercise in the present thesis when compared with moderate-intensity exercise may have resulted in an amplified acute IL-6 response following cycling. In support, the acute increase in plasma IL-6 concentrations was significantly correlated with Tcore at the end of the session, for both
arm-cranking as well as cycling. No correlation was found between final $T_{core}$ and the acute iHsp72 response following arm-cranking or cycling. In corroboration with other studies (Hillman et al., 2011; Morton et al., 2009), this suggests that more factors than hyperthermia alone affect the iHsp72 response to exercise. Indeed, while participants attained a similar $T_{core}$ during cycling (Chapter 2) and HWI (Chapter 5) in the present thesis, only the former resulted in an acute increase in iHsp72 expression.

Chronic training adaptations

Most members of the general population are unaccustomed to upper-body endurance type exercise. As such, findings from studies investigating physiological responses to this modality in able-bodied participants may not necessarily be transferable to people adapted to upper-body exercise, such as wheelchair users. Indeed, comparing the acute iHsp72 response following arm-cranking between paddlers and cyclists, a dampened response was observed in the upper-body trained paddlers (Chapter 2). This corroborates with findings from previous studies showing a dampened acute inflammatory response in trained individuals when compared with untrained individuals (Gokhale et al., 2007). Moreover, chronic training interventions also result in an attenuated acute exercise-induced inflammatory response (Croft et al., 2009; Fischer et al., 2004; Yfanti et al., 2012). In addition to the effect of a higher “overall” physical fitness, the results of Chapter 2 therefore show that local, modality-specific adaptations to exercise training can also dampen the acute inflammatory response to exercise. This may be explained by higher basal glycogen or iHsp72 levels in the chronically trained musculature (Febbraio et al., 2002b; Morton et al., 2009), or the suggested reduction in the oxidative stress response to familiar exercise (Wadley et al., 2016). For instance, exercise in a glycogen-depleted state results in an exacerbated acute inflammatory response (Bishop et al., 2001).

Of note, the results of this thesis do not advocate against chronic exercise training. In contrast, they suggest that individuals adapted to upper-body exercise may need to engage in longer duration or higher intensity exercise to induce the acute elevations in potentially health promoting inflammatory markers. After all, as Morton et al. (2009) suggested: “The exercise protocol must present a novel homeostatic disruption and exceed a `critical threshold’ exercise intensity needed to overwhelm
baseline defence systems in order for a stress response to occur”. From a practical perspective, this may not always be feasible in populations with an already restricted ability to be physically active. In addition, other, disability-specific aspects may further impact on the acute inflammatory response in some individuals adapted to upper-body exercise.

**Autonomic function**

Catecholamines can modulate several aspects of the immune system, including cytokine production by monocytes and NK-cell recruitment into the circulation (Elenkov et al., 2000). The acute elevation of the plasma IL-6 concentration after infusion of adrenaline in resting individuals confirms its importance for the acute response of this marker following exercise (Steensberg et al., 2001). Moreover, blunting the catecholamine response to exercise by clamping $T_{core}$ is associated with smaller elevations of plasma cytokine concentrations following the exercise bout (Rhind et al., 2004). Chapter 3 supports those findings by showing a strong association between catecholamines, adrenaline in particular, and serum IL-6 concentrations in people with SCI following a wheelchair half-marathon. Individuals with a complete CSCI have an impaired autonomic nervous system, hampering the sympathetic innervation of the heart, adrenal medulla and nerve endings below the spinal lesion (Krassioukov, 2006). As a result, the elevation of catecholamines following exercise is dampened or completely blunted in this population. Therefore, the attenuation of this response may play a causal role in the dampened exercise-induced elevation of IL-6 concentrations in people with CSCI observed in Chapter 3 and previous studies (Ogawa et al., 2014; Paulson et al., 2013). Although the impact of autonomic dysfunction on acute inflammatory response to exercise may thus be mediated by catecholamines, the latter is only 1 of the available measures to estimate autonomic function. In Chapter 3, autonomic function at rest was assessed in addition to the acute catecholamine response to exercise. The addition of these resting measures of autonomic function to the regression model increased the explained variance in post-race serum IL-6 concentrations from 60% to 88%, suggesting that the link between autonomic function and the acute IL-6 response is mediated by more than catecholamines only. It may be that the influence of autonomic function on the acute IL-6 response is also partly mediated by its impact on physical capacity (West et al., 2013), and therefore
potentially heat storage during the race (Dill et al., 1931). On the other hand, the impaired thermoregulation during exercise in people with CSCI may result in a relatively higher $T_{\text{core}}$ despite exercising at a lower absolute intensity compared with NON-CSCI wheelchair athletes (Griggs et al., 2014). Hence, continuous $T_{\text{core}}$ monitoring would be an intriguing additional measure in future studies on the potential of upper-body exercise to induce an acute inflammatory response in people with CSCI. In this respect, it is interesting that in contrast to most exercise studies (Kouda et al., 2012; Paulson et al., 2013), HWI can induce an acute IL-6 response in CSCI of similar magnitude compared with able-bodied individuals (Leicht et al., 2014). This suggests that either $T_{\text{core}}$ during exercise is not elevated sufficiently during exercise in people with CSCI, or that the mechanisms underlying IL-6 release into the circulation differ between exercise and HWI.

**Implications for people with a low physical capacity**

Autonomic dysfunction and chronic adaptations in the upper body musculature can dampen the acute inflammatory response to upper-body exercise. Furthermore, a low physical capacity can prevent people engaging in exercise of sufficient volume to elevate the expression of inflammatory markers (Fischer, 2006; Markovitch et al., 2008; Paulson et al., 2014). Additional strategies may therefore be warranted to induce this response in populations with a reduced exercise capacity, such as those with SCI, the elderly or obese individuals. In the present thesis, the efficacy of an alternative upper-body exercise modality (i.e. HIIT) and a passive heating method (i.e. HWI) are explored in this context. In doing so, their applicability in populations restricted from being physically active has been a focus point. Consequently, protocols that could be performed in rehabilitation settings or under free-living conditions in these populations have been investigated. Moreover, as adherence to any health promoting strategy is key to its success, perceptual responses to these interventions have been collected to inform its implementation in practice.
8.2 Strategies to amplify the acute inflammatory response

Upper-body high-intensity interval training

In the last decade, HIIT has gained increased attention as a time-efficient strategy to improve cardiometabolic health in previously sedentary individuals (Gibala et al., 2012). Training studies suggest comparable or larger improvements in cardiometabolic health when compared with traditional continuous moderate-intensity exercise (Fisher et al., 2015). A similar picture can be drawn from studies on the acute inflammatory response following HIIT using the lower-body (Cabral-Santos et al., 2015; Kaspar et al., 2016; Leggate et al., 2010; Peake et al., 2014; Wadley et al., 2016). The present thesis adds to these findings by showing that an upper-body HIIT protocol of 20 min induces similar acute elevations in plasma IL-6 and IL-1ra concentrations compared with moderate-intensity continuous upper-body exercise of 30 min. Of note, the trials in Chapter 4 were matched for total work done. Thus, there seems to be no additional benefit of the high-intensity intervals other than the reduction in time commitment needed for the acute increase in inflammatory markers. This corroborates findings of some (Cabral-Santos et al., 2015; Kaspar et al., 2016; Peake et al., 2014), but not all studies on lower-body exercise (Leggate et al., 2010; Wadley et al., 2016). In the study of Leggate et al. (2010), where a larger acute IL-6 response was observed following HIIT compared with continuous exercise, both sessions were 60 min in duration. Considering the notion that IL-6 is responsive to reduced levels of glycogen stores (Bishop et al., 2001), it may be that the impact of the high-intensity intervals on glycogen depletion is only sufficient to amplify the acute IL-6 response in exercise of longer duration than the protocol employed in Chapter 4. Together, nonetheless, these findings support the notion that the volume of exercise and its resultant energy expenditure is a key component for the magnitude of the acute inflammatory response to exercise (Fischer, 2006). Upper-body HIIT may therefore be a useful additional option for those with limited free time available.

Hot water immersion

In the present thesis, a single HWI session induced an acute elevation of plasma IL-6 and nitrite concentrations, and a change in the distribution of monocyte subsets. This supports the notion that plasma IL-6 can be elevated independently of muscle contraction (Welc et al., 2012). Whether
skeletal muscle is nonetheless the source of the elevated IL-6 concentrations cannot be concluded from the results of the present thesis. However, Welc et al. (2012) showed that passive heating of mice skeletal muscle activates HSF-1 and results in the production of IL-6 and iHsp72, suggesting muscle to function as a “heat sensor”. Another candidate to contribute to circulating IL-6 concentrations are monocytes (Rhind et al., 2002). In Chapter 7, however, no effect of temperature on IL-6 expression in this cell type was observed. Moreover, exercise in the heat does not increase IL-6 expression in monocytes when compared with exercise in thermoneutral conditions (Starkie et al., 2001). Together, this suggests that also in the context of HWI monocytes do not significantly contribute to the increased plasma IL-6 concentrations.

In contrast to IL-6, the ex-vivo model used in Chapter 7 showed that temperature impacts on the iHsp72 expression in monocytes. Incubation for 2 h at 40.0°C almost doubled the iHsp72 expression compared with incubation at 37.0°C; an effect that was absent with incubation at 38.5°C. The latter may explain why no acute iHsp72 response was observed following 1 h of HWI in water set at 39.0°C (resulting in a peak Tcore of 38.7°C) in Chapter 5. This is in line with the acute response observed in skeletal muscle, where 1 h HWI of the leg does not induce an acute iHsp72 response (Morton et al., 2007). On the other hand, 2 h of in-vivo HWI in water set at 39.5°C and incubation of whole blood at 39.0°C elevates iHsp72 expression in monocytes (Oehler et al., 2001). Together, this suggests that large temperature elevations are needed to induce an acute increase in iHsp72 expression in monocytes. This may make HWI, and also exercise for that matter, unfeasible as an intervention to elevate iHsp72 expression in non-athletic populations. The absence of an increase in eHsp72 concentration following a wheelchair half-marathon, and eHsp72 as well as iHsp72 after 90 min of upper-body exercise in people chronically trained in this modality, further support this statement.

Regardless of its potentially ineffectiveness to induce an acute iHsp72 response, the beneficial effects of HWI may be exerted via mechanisms other than the elevation in the expression of this intracellular chaperone. Indeed, the acute elevation of plasma IL-6 concentrations can induce an anti-inflammatory milieu, potentially resulting in an altered expression of inflammatory markers not investigated in this thesis (Gleeson et al., 2011). For instance, regular exercise can reduce TLR
expression on monocytes (Flynn and McFarlin, 2006), increase the proportion of regulatory T-cells in the circulation (Handzlik et al., 2013), shift the macrophage phenotype from predominantly M1 to M2 (Kawanishi et al., 2010), and reduce plasma TNF-α and CRP concentrations while increasing the circulating concentrations of anti-inflammatory cytokines such as IL-10 (Kohut et al., 2006). Whether acute systemic concentrations of IL-6 are indeed a major trigger for these adaptations remains to be determined. The acute increase in plasma nitrite concentration, indicative of increased NO bioavailability, supports the proposed beneficial effects of HWI on the vasculature (Brunt et al., 2016). Moreover, although the evidence is more equivocal, NO may impact on glucose uptake by skeletal muscle; either by the increase in glucose supply to skeletal muscle (Baron et al., 1994) or by directly promoting insulin-independent glucose uptake (Roberts et al., 1997).

To investigate the extent to which the acute responses to a single HWI session lead to chronic adaptations, the effect of a 2-week intervention consisting of 10 HWI sessions on resting metabolic and inflammatory markers was assessed in Chapter 6. Fasting glucose and insulin concentrations were reduced, without changes in resting iHsp72 expression, plasma IL-6 and nitrite concentrations or the monocyte subsets distribution. Resting eHsp72 concentration, however, was reduced following the intervention. These findings, alongside supporting evidence (Chung et al., 2008; Hooper, 1999; Kavanagh et al., 2016), suggest that HWI may improve glucose metabolism. Although elevated iHsp72 expression may lead to improved insulin signalling by e.g. blocking the inflammatory JNK pathway (Chung et al., 2008; Silverstein et al., 2014), the reduction in fasting glucose and insulin occurred independently of changes in the expression of this marker. It may be, therefore, that either iHsp72 measured in monocytes does not reflect the iHsp72 status in the main sites of glucose uptake (i.e. the liver and skeletal muscle), or that changes in iHsp72 are not as crucial for improvements in glucose metabolism as suggested by animal studies (Chung et al., 2008; Silverstein et al., 2014). Moreover, the expression of additional markers implicated in glucose disposal, such as skeletal muscle PGC-1α, can be elevated by chronic passive heating (Hafen et al., 2018). The reduction in fasting glucose and insulin observed in Chapter 6 may thus partly be explained by markers not measured in the present thesis. It should be noted, however, that fasting concentrations of glucose and
insulin are not the gold standard for the measurement of glucose metabolism (Monzillo and Hamdy, 2003). Indeed, it has been proposed that glycaemic responses to a meal are more reflective of metabolic health (Bonora, 2002). Unpublished data from a PhD-thesis show that while fasting glucose and insulin concentrations are reduced following 6 days of mild heat acclimation (i.e. 6 h per day resting in a room set at 36°C), no changes in the response to an oral glucose tolerance test (OGTT) are present (Pallubinsky, 2018). However, although used in research settings, the OGTT was created as a diagnostic tool rather than for research purposes and may not be sensitive enough to detect changes in glucose metabolism following a passive heating intervention (Monzillo and Hamdy, 2003). Nonetheless, future chronic intervention studies on HWI should include assessments of postprandial glycaemic responses, preferably using glycaemic or insulin clamp methods.

The only inflammatory marker that was changed following the chronic HWI intervention period was eHsp72 concentration, indicating a shift to a less inflammatory profile at rest. Indeed, elevated eHps72 concentrations are linked to insulin resistance (Krause et al., 2014) and atherosclerosis (Krepuska et al., 2011). Interestingly, in Chapter 3, there was a trend for higher resting eHsp72 concentrations in wheelchair athletes with CSCI compared with NON-CSCI. As CSCI is associated with an increased risk for chronic low-grade inflammation, this may lend support to consider the reduction in eHsp72 following the chronic HWI intervention of Chapter 6 as a positive outcome. On the other hand, ageing is associated with a reduction in resting eHsp72 concentrations (Njemini et al., 2011) and a chronic HWI intervention in monkeys leads to an increase in resting eHsp72 concentrations alongside an improvement in insulin sensitivity (Kavanagh et al., 2016). Moreover, there seems to be a large day-to-day variability in resting concentrations of eHsp72 (Guy et al., 2017). More research is therefore needed to determine the significance of this marker in the context of chronic low-grade inflammation and cardiometabolic health.

Regardless of the growth in scientific interest in the use of HWI to promote cardiometabolic health (Krause et al., 2015; Brunt et al., 2016), potential safety issues need discussing. To date, the use of HWI and saunas is contra-indicated for people with uncontrolled hypertension, unstable chronic heart disease and dermatological problems (Krause et al., 2015). Survey-based studies, on the
other hand, have reported a low incidence of accidents during passive heating (Laukkanen et al., 2018). Moreover, most of these were associated with the use of alcohol and falls in and around the site were the passive heating session took place (Laukkanen et al., 2018). Nonetheless, the hemodynamic changes during HWI may trigger a cardiac event in at-risk populations. For instance, Chiba et al. (2005) observed cardiac arrhythmia during HWI in a few of the elderly participants. In people already suffering from cardiac arrhythmia, HWI may exacerbate these symptoms and put one at risk for a cardiac event. In addition, the lowered blood pressure during HWI increases the risk for orthostatic hypotension, especially when standing up to leave the hot tub. Collectively, although there is a lack of longitudinal research into the potential health and safety hazards surrounding passive heating, it is prudent to contra-indicate the use of passive heating interventions in the populations described above as well as to warn people for the risk of orthostatic hypotension when leaving the hot tub.

8.3 Perceptual responses to exercise and hot water immersion

The perceptual responses to a certain activity are suggested to be important in the decision to repeatedly engage in that particular activity (Ekkekakis et al., 2011). Understanding the potential factors that influence these responses during exercise and HWI may therefore aid to increase adherence to such health promoting strategies. Perceptual responses have been collected throughout this thesis, with a focus on basic affect and ratings of enjoyment. Chapter 2 showed that perceptual responses are not affected by familiarity to the exercise modality. This suggests that having to change exercise modality due to injury or acute disability does not lead to more negative perceptual responses to the newly adopted exercise mode. While the affective responses during HIIT in Chapter 4 were more negative compared with moderate-intensity exercise, the inclusion of intervals during exercise enhanced ratings of enjoyment reported after the exercise session. Thus, although HIIT is not necessarily more effective in inducing an acute inflammatory response, it may be advised to include intervals during upper-body exercise to promote regular engagement in exercise. Moreover, the similar ratings of enjoyment between moderate-intensity intervals (i.e. CAD) and high-intensity intervals (i.e. HIIT) suggest that intervals do not have to be of high-intensity to enhance enjoyment.
Promisingly, however, also high-intensity intervals seem to be well tolerated by people with SCI and lead to higher ratings of enjoyment compared with continuous exercise in this population (Astorino and Thum, 2016). Notwithstanding these findings, and considering the negative affective responses during HIIT (Hoekstra et al., 2017; Jung et al., 2014; Kilpatrick et al., 2015), it is yet to be determined what is a more important driver for adherence: affective responses during exercise or ratings of enjoyment reported after exercise.

If HWI were to be implemented as a health promoting tool in rehabilitation settings or free-living environments, the perceptual responses to this intervention may need further investigation. Indeed, the affective responses near the end of a HWI session were similar compared with upper-body HIIT, and considerable thermal discomfort was reported. Of note, as observed during exercise (Ekkekakis et al., 2011), there was a large inter-individual variability in the affective responses during HWI. This suggests that some, but not all individuals perceive this activity as unpleasant. It is promising that in Chapter 6 of the present thesis only 1 out of 11 sedentary, overweight participants dropped out during the intense 2-week chronic HWI intervention. Nonetheless, future work should investigate alternative HWI protocols to enhance the perceptual responses to this activity, while at the same time ensuring its effectiveness to drive positive adaptations in cardiometabolic health. Examples of interventions that may enhance the perceptual responses are shortening of the protocol, including short breaks during the session, or applying local cooling to alter thermal perceptions.

8.4 Limitations and recommendations for future research

In line with any research project, the experiments conducted as part of this thesis have several limitations and have left opportunities for future research. Notably, the novel findings presented on the acute and chronic effects of HWI on metabolic and inflammatory markers provide strong rationale to further investigate its potential to be used as an additional or alternative strategy to promote health in people restricted to be physically active.
Factors influencing the acute inflammatory response to exercise

Chapter 2 and 3 have investigated factors that may influence the acute inflammatory response to exercise. Although the main aim of Chapter 2 was to investigate the impact of chronic modality-specific training adaptations, better matching of the relative exercise intensity between the upper- and lower-body exercise protocols would have provided more in-depth understanding of the impact of active muscle mass on the anti-inflammatory effects of prolonged interval exercise. As exercise intensity was matched for relative PO rather than \( \dot{V}O_2 \), the latter was not controlled during the sessions. This approach was taken as the intermittent character of the exercise makes matching for relative \( \dot{V}O_2 \) problematic due to the time it takes for this parameter to reach a steady state (Whipp and Wasserman, 1972). As a result, although the relative external workload (i.e. PO) was matched between arm-cranking and cycling, the latter was performed at a higher relative internal workload (i.e. \( \dot{V}O_2 \)). Furthermore, the relatively high absolute intensities during arm-cranking in both groups, makes it difficult to extrapolate the findings of this study to people with a low physical capacity, such as those with CSCI.

Although the relative internal workload differed between upper- and lower-body exercise in Chapter 2, the difference in T\(_{\text{core}}\) between both modalities may partly explain the lower acute inflammatory response following arm-cranking. To further investigate the potential of body temperature manipulations to enhance the acute inflammatory response to upper-body exercise in people with a low physical capacity, future studies may consider manipulating body temperature during upper-body exercise, such as done in Rhind et al. (2004) in lower-body exercise. The findings from Leicht et al. (2016) suggest that it is particularly important to manipulate T\(_{\text{core}}\), as increases in T\(_{\text{skin}}\) do not seem to significantly impact on the acute inflammatory response to upper-body exercise. To further assess the influence of autonomic function on IL-6 production in response to exercise, future studies could include CSCI wheelchair athletes only, with varying levels of autonomic completeness of the lesion. This would partly mitigate the effect of lesion level that is inherently present when comparing people with CSCI and paraplegia. Finally, the physiological underpinning of heart rate and blood pressure variability measures in the frequency domain used to assess autonomic
function at rest in Chapter 3 have been the subject of debate (Billman, 2013). While it is indeed questionable whether LF HRV and LF SBP reflect solely sympathetic activation (Billman, 2013), the measures included in Chapter 3 strongly correlate with more clinical outcomes of autonomic dysfunction (Claydon & Krassioukov, 2008). Moreover, these measures have been shown reliable in people with SCI (Ditor et al., 2005). Nevertheless, these measures should be considered as autonomic function indices, rather than measures of sympathetic activation (Esco et al., 2018). Finally, it has recently been shown that psychological stress and anxiety prior to exercise can influence subsequent immune response following the exercise bout (Edwards et al., 2018). Although no evidence for such effect exists for the markers included in the present thesis, the omission of control for this aspect may be regarded as a potential limitation.

**Health promoting effects of hot water immersion**

The experiments on HWI described in Chapter 5 and 6 had several potential limitations. Firstly, resting in a room at ambient temperature was chosen as a control condition above immersion in thermoneutral water. Therefore, the effect of hydrostatic pressure on any of the outcome measures cannot be excluded. However, Laing et al. (2008) suggests that the effect of this on inflammatory markers is negligible. Moreover, in this thesis the focus was on HWI as a stand-alone health promoting intervention rather than to investigate the effects of independent body temperature elevations. Nevertheless, the practical focus of this thesis may have hindered some mechanistic understanding that could have been gained from these studies. The same applies to the control condition employed in the chronic intervention study described in Chapter 6, in which participants refrained from any type of intervention rather than engaging in immersion in thermoneutral water.

Regarding the outcome measures included in Chapter 5 and 6, 2 aspects need consideration. Firstly, considering the variation in peak values observed in iHsp72 expression following acute exercise interventions, it may be that the peak in iHsp72 expression following HWI occurred after the 2 h post-HWI blood sample in Chapter 5. For instance, while Fehrenbach et al. (2001), Hillman et al. (2011) and Febbraio et al. (2002) observed a peak in iHsp72 expression immediately post exercise, peak post-exercise values are observed at 30 min, 90 min (Peart et al., 2013), 24 h (Périard et al.,
Moreover, Hafen et al. (2018) failed to observe an acute increase in iHsp72 in muscle biopsies taken immediately following 2 h of local passive heating, while in the same study a short-term chronic intervention led to an elevated resting iHsp72 expression.

Secondly, as mentioned before, fasting glucose and insulin are not the gold standard for the assessment of glucose metabolism. To gain more understanding in the potential of HWI to improve glucose metabolism/insulin sensitivity, future studies should use more sophisticated methods, such as a euglycemic clamp. To investigate the significance of iHsp72 in potential HWI-induced improvements in glucose metabolism in humans, future studies should consider simultaneous assessments of its expression in insulin sensitive tissue such as skeletal muscle and adipose tissue, as well as circulating monocytes. Moreover, a strong correlation between iHsp72 expression in the main site of glucose disposal (i.e. skeletal muscle) and monocytes would further support the use of the latter as a surrogate measure for whole body iHsp72 expression. Considering the relative ease and less intrusive nature of blood sampling compared with the collection of tissue biopsies, this would make studies into the influence of iHsp72 on metabolic health in humans more accessible. In turn, as the duration of the chronic intervention in Chapter 6 was relatively short, this may stimulate work on longer-term interventions to follow up on the findings presented in this thesis and further inform the implementation of this intervention.

**Applicability of the findings**

Studies investigating methods to amplify the acute inflammatory response to upper-body exercise and alternative health promoting strategies are arguably most relevant for people with a restricted ability to engage in (lower-body) exercise. The relatively high physical capacity of the participants investigated in most experiments of this thesis may therefore be considered a limitation. However, studies into potential influences on the acute inflammatory response to upper-body exercise require a relatively homogeneous sample. The inclusion of (novice) able-bodied individuals was therefore preferred over a heterogeneous group of people with varying disabilities. Nevertheless, follow-up studies should investigate whether the findings of the present thesis are transferable to
wheelchair users, the elderly or obese individuals. For instance, it would be interesting to follow up on the observation of a dampened acute inflammatory response to upper-body exercise in people accustomed to this modality, as observed in Chapter 2, by longitudinally monitoring this acute response in wheelchair users that have entered their post-injury rehabilitation phase. In addition, although upper-body HIIT is well tolerated in people with paraplegia (Astorino et al., 2016), it is not known whether the lower anaerobic capacity of individuals with CSCI (Dallmeijer et al., 1994) affects the feasibility of this form of exercise in this group. Finally, several populations may benefit from HWI interventions. While passive heating may be suitable and safe even for those with stable CVD (Laukkanen et al., 2018), more research is needed into how well this intervention is tolerated by individuals with a low physical capacity. The inclusion of measurements of perceptual responses in such studies would be key to inform its implementation in practice.

8.5 Conclusion

The present thesis sought to investigate factors that may influence the acute inflammatory response to exercise as well as the efficacy of additional or alternative strategies to induce this response. A reduced active muscle mass as well as chronic modality-specific training adaptations can reduce the acute inflammatory response to interval upper-body exercise. Moreover, autonomic dysfunction may further attenuate the potential of upper-body exercise to induce acute elevations in plasma IL-6 concentrations. As a potential alternative or additional health promoting strategy, upper-body HIIT may be a more time-efficient and enjoyable form of exercise to induce acute elevations in inflammatory markers compared with continuous moderate-intensity upper-body exercise. Furthermore, HWI can induce an acute inflammatory response independent of muscle contraction. Together with the reduced fasting glucose, insulin and eHsp72 concentrations following a 2-week intervention, this suggests that this applicable form of passive heating may be a viable tool to reduce chronic low-grade inflammation and improve cardiometabolic health in people restricted to be physically active.
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Appendices

Appendix 1 Participant informed consent form
Appendix 2 Perceptual scales
Appendix 3 Description of exercise ergometers and immersion tank
Appendix 4 Staining protocol for flow cytometric analyses
Appendix 5 Gating strategy for determination of iHsp72 expression and monocyte subset distribution
Appendix 1 Participant informed consent form

Perceptual responses for different exercise modalities
INFORMED CONSENT FORM
(to be completed after Participant Information Sheet has been read)

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

Yes ☐ No ☐

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

Yes ☐ No ☐

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

Yes ☐ No ☐

I understand that I am under no obligation to take part in the study.

Yes ☐ No ☐

I understand that I have the right to withdraw from this study at any stage for any reason, and that I will not be required to explain my reasons for withdrawing.

Yes ☐ No ☐

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

Yes ☐ No ☐

I agree to participate in this study.

Yes ☐ No ☐

I agree that the bodily samples taken during this study can be stored for future research. All samples will be destroyed after a maximum of five years.

Yes ☐ No ☐

If No to above, I confirm that the bodily samples taken during this study can only be used for this study and should be disposed once the inflammatory markers as described in the participant information sheet have been analysed.

Yes ☐ No ☐

Your name

______________________________

Your signature

______________________________

Signature of investigator

______________________________

Date

______________________________
## Appendix 2 Perceptual scales

1. **LOW AROUSAL**

2

3

4

5

6. **HIGH AROUSAL**

<table>
<thead>
<tr>
<th></th>
<th>Feeling Scale</th>
</tr>
</thead>
<tbody>
<tr>
<td>+5</td>
<td>Very Good</td>
</tr>
<tr>
<td>+4</td>
<td>Good</td>
</tr>
<tr>
<td>+3</td>
<td>Fairly Good</td>
</tr>
<tr>
<td>+2</td>
<td>Neutral</td>
</tr>
<tr>
<td>+1</td>
<td>Fairly Bad</td>
</tr>
<tr>
<td>0</td>
<td>Bad</td>
</tr>
<tr>
<td>-1</td>
<td></td>
</tr>
<tr>
<td>-2</td>
<td></td>
</tr>
<tr>
<td>-3</td>
<td></td>
</tr>
<tr>
<td>-4</td>
<td></td>
</tr>
<tr>
<td>-5</td>
<td>Very Bad</td>
</tr>
</tbody>
</table>

Felt Arousal Scale  
Svebak & Murgatroyd, 1985

<table>
<thead>
<tr>
<th>6</th>
<th>No exertion at all</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>Extremely light</td>
</tr>
<tr>
<td>8</td>
<td>Very light</td>
</tr>
<tr>
<td>9</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>Light</td>
</tr>
<tr>
<td>12</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>Somewhat hard</td>
</tr>
<tr>
<td>14</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>Hard (heavy)</td>
</tr>
<tr>
<td>16</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>Very hard</td>
</tr>
<tr>
<td>18</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>Extremely hard</td>
</tr>
<tr>
<td>20</td>
<td>Maximal exertion</td>
</tr>
</tbody>
</table>

The Borg 6-20 scale  
(Borg et al., 1987)
<table>
<thead>
<tr>
<th>Thermal Comfort Scale</th>
<th>Thermal Sensation Scale</th>
</tr>
</thead>
<tbody>
<tr>
<td>+3. Uncomfortable</td>
<td>8. Hot</td>
</tr>
<tr>
<td>+2. Slightly uncomfortable</td>
<td>7. Warm</td>
</tr>
<tr>
<td>+1.</td>
<td>6. Slightly warm</td>
</tr>
<tr>
<td>0. Comfortable</td>
<td>5. Neutral</td>
</tr>
<tr>
<td>-1.</td>
<td>4. Slightly cool</td>
</tr>
<tr>
<td>-2. Slightly uncomfortable</td>
<td>3. Cool</td>
</tr>
<tr>
<td>-3. Uncomfortable</td>
<td>2. Cold</td>
</tr>
<tr>
<td>-4. Very uncomfortable</td>
<td>1. Very cold</td>
</tr>
</tbody>
</table>

Thermal Comfort Scale  
(Gagge et al., 1967)  
Thermal Sensation Scale  
(Gagge et al., 1967)
<table>
<thead>
<tr>
<th>I enjoy it</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>I hate it</th>
</tr>
</thead>
<tbody>
<tr>
<td>I feel bored</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
<td>I feel interested</td>
</tr>
<tr>
<td>I dislike it</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
<td>I like it</td>
</tr>
<tr>
<td>I find it pleasurable</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
<td>I don’t find it pleasurable</td>
</tr>
<tr>
<td>I am very absorbed in this activity</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
<td>I am not at all absorbed in this activity</td>
</tr>
<tr>
<td>It’s no fun at all</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
<td>It’s a lot of fun</td>
</tr>
<tr>
<td>I find it energizing</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
<td>I find it tiring</td>
</tr>
<tr>
<td>It makes me depressed</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
<td>It makes me happy</td>
</tr>
<tr>
<td>It’s very pleasant</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
<td>It’s very unpleasant</td>
</tr>
<tr>
<td>I feel good physically while doing it</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
<td>I feel bad physically while doing it</td>
</tr>
<tr>
<td>It’s very invigorating</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
<td>It’s not at all invigorating</td>
</tr>
<tr>
<td>I am very frustrated by it</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
<td>I am not at all frustrated by it</td>
</tr>
<tr>
<td>It’s very gratifying</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
<td>It’s not at all gratifying</td>
</tr>
<tr>
<td>It’s very exhilarating</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
<td>It’s not at all exhilarating</td>
</tr>
<tr>
<td>It’s not all stimulating</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
<td>It’s very stimulating</td>
</tr>
<tr>
<td>It gives me a strong sense of accomplishment</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
<td>It doesn’t give me a strong sense of accomplishment</td>
</tr>
<tr>
<td>It’s very refreshing</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
<td>It’s not at all refreshing</td>
</tr>
<tr>
<td>I felt as though I would rather be doing something else</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
<td>I felt as though there is nothing else I would rather be doing</td>
</tr>
</tbody>
</table>

Physical Activity Enjoyment Scale
(Kendzierski & De Carlo, 1991)
Appendix 3 Description of exercise ergometers and immersion tank

Lode arm-crank ergometer used in Chapters 2 and 4

Water immersion tank used in Chapters 5 and 6

Lode cycle ergometer used in Chapter 2
Appendix 4 Staining protocol for flow cytometric analyses

Slight modifications to this protocol are described in the respective chapters.

<table>
<thead>
<tr>
<th>Antibody/Isotype control</th>
<th>Conjugate</th>
<th>Volume</th>
<th>Manufacturer</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Unstained</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. REA Control</td>
<td>FITC</td>
<td>4 µl</td>
<td>Miltenyi</td>
</tr>
<tr>
<td>CD16</td>
<td>PE</td>
<td>2.5 µl</td>
<td>Miltenyi</td>
</tr>
<tr>
<td>CD14</td>
<td>PerCP</td>
<td>5 µl</td>
<td>Miltenyi</td>
</tr>
<tr>
<td>3. Anti-HSP70</td>
<td>FITC</td>
<td>4 µl</td>
<td>Miltenyi</td>
</tr>
<tr>
<td>CD16</td>
<td>PE</td>
<td>2.5 µl</td>
<td>Miltenyi</td>
</tr>
<tr>
<td>CD14</td>
<td>PerCP</td>
<td>5 µl</td>
<td>Miltenyi</td>
</tr>
</tbody>
</table>

1. Switch on centrifuge and bring PBS to room temperature  
2. Pipette the above volumes of antibodies, isotype controls and Fc block into the tubes. Pipette on to the side near the bottom of the FACS tube.  
3. Add 60 µl of blood to the tubes.  
4. Vortex the tubes.  
5. Incubate in the dark at room temperature for 15 minutes.  
6. Add 750 µl FACS lysis solution at room temperature  
7. Vortex the tubes  
8. Incubate in the dark at room temperature for 10 minutes.  
9. Add 1.5 ml of PBS containing 0.1% BSA and 2mM EDTA.  
10. Centrifuge at 400g for 5 minutes at 20°C.  
11. Tip out supernatant and blot on tissue.  
12. Add 60 µl of FIX (reagent A)  
13. Vortex the tubes  
15. Add 1.5 ml PBS containing 0.1% BSA and 2mM EDTA.  
16. Vortex the tubes  
17. Centrifuge at 400g for 5 min at 20°C.  
18. Tip out supernatant and blot on tissue.  
19. Add 60 µl of PERM (reagent B)  
20. Add the HSP antibodies and isotype controls to tubes 2 and 3.  
21. Vortex the tubes  
22. Incubate for 30 minutes at room temperature in the dark.  
23. Add 2.25 ml of PBS to all tubes  
24. Centrifuge at 400g for 5 min at 20°C.  
25. Tip out supernatant and blot on tissue.  
26. Re-suspend cells in 500 µl PBS containing 0.1% BSA and 2mM EDTA.  
27. Vortex prior to running the samples.
**Appendix 5** Gating strategy for determination of iHsp72 expression and monocyte subset distribution

Gating strategy used in Chapters 2, 5, 6 and 7. CD14 positive cells were selected after exclusion of CD56 positive natural killer cells (the latter only in Chapter 7). Thereafter, the distribution of monocyte subsets based on CD16 and CD14 expression as well as iHsp72 expression was determined.

* Exclusion of CD56 positive cells was only performed in Chapter 7.