**Study Title: *To bathe, or not to bathe?* The effect of acute, passive heating on glucose tolerance in individuals with type 2 diabetes mellitus: a randomised, balanced crossover, control trial.**

**NCT03798717**

**Ethics Ref: 18/SC/0581**

**Date and Version No: 1.2 05-04-2019**

| | |
|---|---|
| Chief Investigator: | Dr. Anthony Shepherd |
| Investigators: | See protocol development team below |
| Sponsor: | Portsmouth Hospitals NHS Trust |
| Funder: | University of Portsmouth |
| Signatures: |  |

Protocol Development Team:

| | | |
|---|---|---|
| Chief Investigator | - Dr Anthony Shepherd | (University of Portsmouth) |
| Principal Investigator | - Mr Thomas James | (University of Portsmouth) |
| Co- investigator 1 | - Dr Jo Corbett | (University of Portsmouth) |
| Co- investigator 2 | - Dr Joseph Costello | (University of Portsmouth) |
| Co- investigator 3 | - Prof. Mike Tipton | (University of Portsmouth) |
| Co- investigator 4 | - Prof. Janis Shute | (University of Portsmouth) |
| Co- investigator 5 | - Dr Zoe Saynor | (University of Portsmouth) |
| Co- investigator 6 | - Dr. Iain Cranston | (Queen Alexandra Hospital) |
| Co- investigator 7 | - Prof. Michael Cummings | (Queen Alexandra Hospital) |
| Co- investigator 8 | - Mr Connor Morgan | (University of Portsmouth) |
| Co- investigator 9 | - Mr Billy Hopkins | (University of Portsmouth) |
| Co- investigator 10 | - Miss Emily Windsor | (University of Portsmouth) |
| Co-investigator 11 | -Ms Sharon Allard | (Queen Alexandra Hospital) |

## TABLE OF CONTENTS

## 1. AMENDMENT HISTORY

| Amendment No. | Protocol Version No. | Date issued | Author(s) of changes | Details of Changes made |
|---|---|---|---|---|
| 1 | 1 | n.a | Dr Shepherd | Changes requested by REC. Please see letter in site file. |
| 2 | 1.2 | | Dr Shepherd | Added research nurse and recruitment via primary care. |

## 2. SYNOPSIS

| | |
|---|---|
| **Study Title** | *To bathe, or not to bathe*. The effect of acute, passive heating on glucose tolerance in individuals with type 2 diabetes mellitus: a randomised, balanced crossover, control trial. |
| **Internal ref. no.** | N/A |
| **Problem statement** | Type 2 diabetes mellitus (T2DM) is characterised by chronic high blood sugar concentration (hyperglycaemia) and insulin resistance leading to a reduction in insulin sensitivity. These hyperglycaemic excursions can seriously impact metabolic, micro and macrovascular health. The total cost of the direct and indirect care of individuals with diabetes (~90% T2DM) in the UK is £23.7 billion, equating to ~20% of the annual national health service (NHS) budget, with this projected to become unsustainable. Low-cost interventions to improve glycaemic control in these individuals are therefore warranted. Current interventions include pharmaceuticals, exercise and calorie restrictive diets. Pharmaceutical interventions carry a high financial cost, while exercise and diet programmes have a low adherence rate in individuals with T2DM.<br><br>Heat therapy offers one potential low cost therapy. Immersion in a hot tub for 30 mins.day$^{-1}$ for 10 days has been shown to reduce fasting plasma [glucose] and HbA1c in individuals with T2DM, which may be explained by acute (e.g. muscle) and chronic (e.g. reduced inflammatory status and increased heat shock proteins (HSP)) adaptations, although experimental evidence for these hypothesis are sparse. Other potential benefits include improved glycaemic control, insulin sensitivity, elevated resting metabolic rate and improved micro- and macrovascular function.<br><br>The aim of the present study is to determine whether acute hot water immersion can improve glucose tolerance in individuals with T2DM and whether it is more beneficial to undertake this before or after a OGTT. |
| **Research question / hypothesis** | *In individuals with type 2 diabetes mellitus:*<br><br>*Primary question:*<br>1. Does an acute bout of passive, warm water therapy reduce plasma [glucose]?<br><br>*Secondary questions:*<br>*1.* Does plasma [glucose] reduce more if the passive, warm water therapy is conducted before or after the OGTT?<br>*2.* Does plasma [insulin] reduce more if the passive, warm water therapy is conducted before or after the OGTT? |

| | |
|---|---|
| | 3. Does insulin sensitivity increase following an acute bout of warm water therapy?<br>4. Does fuel utilisation alter during and following an acute bout of warm water therapy?<br>5. Does cardiovascular status change during or after an acute bout of warm water therapy?<br>6. Does eHSP increases during and following an acute bout of warm water therapy?<br>7. Does inflammatory status change during or after an acute bout of warm water therapy? |
| **Study Design** | Randomised, balanced crossover trial, with 3 conditions:<br><br>1) OGTT in thermoneutral air (~ 23°C air),<br><br>2) Passive heating after an OGTT<br><br>and<br><br>3) Passive heating before OGTT<br><br>For conditions 2 and 3, $T_{rec}$ will be clamped at ~38.5°C using warm water. |
| **Study Participants** | Adults ≥ 35 years, diagnosed with T2DM as defined by the world health organisation (WHO) |
| **Planned Sample Size** | We aim to recruit 20 individuals with T2DM. |
| **Follow-up duration** | 7 days |
| **Planned Study Period** | 18 months |
| **Primary Objective** | To determine whether an acute bout of passive, warm water therapy reduces area under the curve (AUC) [glucose] in individuals with T2DM during an OGTT? |
| **Secondary Objectives** | *In individuals with type 2 diabetes mellitus:*<br><br>1. Does plasma [glucose] reduce more if the passive, warm water therapy is conducted before or after the OGTT?<br>2. Does plasma [insulin] reduce more if the passive, warm water therapy is conducted before or after the OGTT?<br>3. Does insulin sensitivity increase following an acute bout of warm water therapy?<br>4. Does fuel utilisation alter during and following an acute bout of warm water therapy?<br>5. Is cardiovascular status altered during or after an acute bout of warm water therapy? |

| | |
|---|---|
| | 6. Does eHSP increases during an acute bout of warm water therapy?<br>7. Does inflammatory status reduce during or after an acute bout of warm water therapy? |
| **Primary Endpoint** | To determine whether an acute bout of passive, warm water therapy reduces area under the curve (AUC) [glucose] in individuals with T2DM during an OGTT? |
| **Secondary Endpoints** | All other endpoints, including: plasma [insulin], insulin sensitivity, substrate oxidation, [eHSP70], inflammatory markers and cardio vascular measures (e.g. heart rate, blood pressure). |
| **Intervention (s)** | Acute (60 min) bouts of warm water therapy aiming to clamp $T_{rec}$ at 38.5°C (~39°C water temperature, ~ 90%RH).. |

## 3. ABBREVIATIONS

| | |
|---|---|
| AE | Adverse event |
| ANOVA | Analysis of variance |
| AUC | Area under the curve |
| CF | Cystic fibrosis |
| CGM | Continuous glucose monitoring |
| CPET | Cardiopulmonary exercise test |
| CRF | Clinical records file |
| DOVE | Dysglycaemia, Oxidative stress and the Vascular Endothelium |
| ECG | Electrocardiogram |
| EDTA | Ethylenediaminetetraacetic acid |
| eGFR | Estimated glomerular filtration rate |
| eHSP | Extracellular heat shock protein |
| GCP | Good clinical practise |
| Hb | Haemoglobin |
| $HbA1_c$ | Haemoglobin adult type $1_c$ (Glycated haemoglobin) |
| Hct | Haematocrit |
| HEC | Hyperinsulinaemic-euglycaemic clap |
| HIIT | High intensity interval training |
| HR | Heart rate |
| HRV | Heart rate variability |
| HSP | Heat shock protein |
| iHSP | Intracellular heat shock protein |
| IL | Interleukin |
| INM | Institute of naval medicine |
| LF | Low frequency |
| NHS | National Health Service |
| NIHR | National institute for health research |
| NIRS | Near infrared spectroscopy |
| NO | Nitric oxide |
| NRES | National research ethics service |

| | |
|---|---|
| OGIS | oral glucose insulin sensitivity |
| OGTT | Oral glucose tolerance test |
| PCPI | Patient, carer and public involvement |
| PPI | Patient public involvement |
| QUICKI | Quantitative insulin sensitivity check index |
| REC | Research ethics committee |
| RMR | Resting metabolic rate |
| RMSSD | Root mean square of the successive differences |
| RSA | Respiratory sinus arrhythmia |
| SAE | Serious adverse event |
| T2DM | Type 2 diabetes mellitus |
| TNF-α | Tumour necrosis factor alpha |
| $T_{rec}$ | Rectal temperature |
| UoP | University of Portsmouth |
| WHO | World health organisation |

## 4. BACKGROUND AND RATIONALE

Current estimates suggest 422 million people worldwide live with a form of diabetes, of which ~ 90% have T2DM [WHO, 2016]. The total cost of the direct and indirect care of individuals with diabetes in the UK is £23.7 billion, equating to ~ 20% of the annual NHS budget [Hex et al., 2012], this figure is expected to rise to £39.8 billion by the year 2035 [Hex et al., 2012; Shaw et al., 2010]. Approximately 85-90% of cases of T2DM arise from a poor lifestyle and obesity [ERFC, 2010; George et al., 2013; Kelly et al., 2008], with the other 10-15% resulting from genetic predispositions [Daousi et al., 2006; DPPRG, 2002]. Current interventions include pharmaceutical treatments, exercise and calorie restrictive diets, which aim to improve glycaemic control. However, pharmaceutical interventions carry a high financial cost [Cheng and Fantus, 2005; Nathan et al., 2006], while exercise and diet programmes have a low adherence rate in individuals with T2DM [Ary et al., 1986; Barnard et al., 2000; Clark, 1997; Glasgow et al., 1997; Nathan et al., 2006; Stunkard and Messick, 1985]. With the population of individuals with T2DM continuing to increase, the costs associated with the clinical care of these individuals are likely to become unsustainable. Simple, inexpensive interventions to improve the clinical profile of this group are therefore needed.

One such strategy could be passive heating. Preliminary evidence suggests passive heating may have beneficial effects for metabolic health in animal models [Bathaie et al., 2010; Chung et al., 2008; Gupte et al., 2009; Gupte et al., 2010; Kokura et al., 2007; Morino et al., 2008; ] and in humans [Chung et al., 2008; Faulkner et al., 2017; Hooper, 1999; Rivas et al., 2016; ]. This review will focus on the use of warm water and air, and proposed mechanisms of actions for these therapeutic aids in individuals with T2DM.

In 1999, the use of hot tubs (38-41°C , 30 mins / day for 3 weeks) was shown to reduce fasting plasma glucose concentrations by ~ 14% (1.3 $mmol.L^{-1}$) and decrease $HbA_{1c}$ by ~10-11 mmol/mol in 8 adults with T2DM [Hooper, 1999]. Given the rate of turnover in haemoglobin this reduction is surprising as the treatment period was run over 3 weeks and the total haemoglobin turnover takes ~115 days [Cohen et al., 2008]. Rivas et al., (2016) is the only other study to date that has investigated how passive heating effects glycaemic control in individuals with T2DM since Hooper (1999). In contrast, Rivas et al. (2016) showed no significant difference in plasma glucose concentration during a 120 min OGTT 24 h after a passive heating bout in the water compared to a normothermic control condition. To date, the effects of acute passive heating in close proximity to an OGTT in individuals with T2DM has yet to be investigated. However, there has been promising evidence from healthy volunteers, showing that passive heating may be just as good or even better at reducing hyperglycaemia than exercise [Faulkner et al., 2017]. Faulkner et al. (2017) showed that compared to exercise, passive heating reduced peak plasma [glucose] significantly more and AUC plasma [glucose] the same amount. Additionally, there has been an abundance of animal studies showing beneficial effects on glycaemic control that have investigated the effect of passive heating [Bathaie et al., 2010; Chung et al., 2008; Gupte et al., 2009; Gupte et al., 2010; Kokura et al., 2007; Morino et al., 2008]. Together, these results show that passive heating in close proximity

to a glucose challenge has the potential to improve glucose tolerance, insulin-stimulated glucose uptake and increase insulin signalling in individuals with T2DM.

In contrast to warm water and its purported increased glucose disposal [Faulkner et al., 2017; Hooper, 1999], warm ambient conditions have previously been shown to increase plasma glucose concentrations. For example, plasma glucose levels increase after a 120 min OGTT in temperatures of 30-35℃compared to 20-25 in healthy young individuals [Moses et al., 1997]. This has also been shown in individuals with diabetes (obese and non-obese) and health obese and non-obese [Akanji & Oputa, 1991; Akanji & Oputa, 1991]. Obesity spears to play a key role in the change in glucose concentration, driven by an increased heat conduction time [Akanji & Oputa, 1991].

Proposed mechanisms for this include, increasing cutaneous blood flow, subsequently reducing visceral blood flow and therefore reducing glucose disposal from the blood [Frayn et al., 1989; Moses et al., 1997]. However, increases in ambient air temperature are unlikely to increase core temperature over long periods when ambient air temperature is ≤ 28℃ [Haldane, 1905]. Whist increases in core temperature have been strongly linked with increases in visceral [Rowell et al., 1968; Rowell., 1970; Rowell et al., 1971] and skeletal muscle blood flow [Crandall et al., 2008; Heinonen et al., 2011]. Importantly, when there is a significant increase in ambient air temperature muscle both muscle and liver blood flow are reduced [Rowell 1968]. Conversely, an increase in core temperature will perfuse visceral [Rowell et al., 1968 Rowell., 1970; Rowell et al., 1971] and skeletal muscle tissue [Crandall et al., 2008; Heinonen et al., 2011], which are key sites for glucose disposal and may have a beneficial effect.

Hooper (1999) postulated that an increase in skeletal muscle blood flow may be responsible for this increased glucose clearance, citing evidence that this can modulate insulin mediated glucose uptake [Baron et al., 1994]. Other mechanisms have also been suggested, but have yet to be confirmed, including; increased insulin sensitivity [Hermanns et al., 2014; Raz et al., 2009], altered inflammatory response [Hundal et al., 2002; Shoelson et al., 2003; Yuan et al., 2001] and activation of heat shock proteins (HSP) [Rodrigues-Krause et al., 2012]. Each potential mechanism is discussed below:

1) <u>Increased skeletal muscle and liver blood flow:</u>

Since the hypothesis that heating may cause an elevation in blood flow and increase glucose clearance, several studies have looked to examine the effect of heating on blood flow. Using local heating, whole body heating and water perfused suites, Crandall et al., (2008) and Heinonen et al., (2011) showed that passive heating increases both muscle blood flow and cardiac output. This may be at least in part due to passive heating raising core temperature, therefore increasing cardiac output and also muscle blood flow in an attempt to lose heat. Interestingly, Baron et al. (1994) showed that an increase in plasma insulin caused a significant increase in leg muscle blood flow from ~0.15 $L \cdot min^{-1}$ to 0.35 $L \cdot min^{-1}$. Insulin is produced for the

vasodilatory response, which aids increased blood flow and maintains blood pressure [Baron et al., 1994; Heinonen et al., 2011].

This increase in cardiac output and skin blood flow is not dissimilar to when individuals exercise in the heat [Braun et al., 1995; Brunt et al., 2016; Crandell et al., 1999; Faulkner et al., 2017; Heinonen et al., 2011; Rivas et al., 2016; Voulgari et al., 2013]. Therefore, passive heating may mimic the beneficial effects on glucose homeostasis seen following exercise. While this evidence supports a rise in muscle blood flow during heating, there is no evidence as to how long blood flow stays elevated post heating without actively cooling individuals.

2) Passive heating as an exercise mimetic

Multiple studies have examined the effect of exercise-induced hyperthermia and glucose homeostasis [Braun et al., 1995; Caldwell et al., 2011; Faulkner et al., 2017; Gibson et al., 2014; Jorge et al., 2011; Kadoglou et al., 2007; Kang et al., 1996; O'gorman et al., 2006; Periard et al., 2011; Voulgari et al., 2013]. All of these studies show that exercise improves insulin sensitivity irrespective of the aerobic exercise programme that was used. Acute aerobic exercise increases muscle glucose uptake five-fold via insulin-independent mechanisms [Roberts et al., 2013] and even a single bout of high-intensity exercise can improve insulin sensitivity [Cockcroft et al., 2018]. Post-exercise, glucose uptake remains elevated via both insulin-independent (~120 min) and insulin-dependent (up to 48 h) mechanisms if exercise is prolonged [Magkos et al., 2008]. Improvements may be seen in insulin sensitivity post exercise after just 20 min of high-intensity exercise, lasting for ~24 h [Gillen et al., 2012; Manders et al., 2010]. Low-intensity aerobic exercise (≥60 min) can enhance insulin sensitivity in obese, insulin –resistant adults for at least 24 h post exercise [Newsom et al., 2013]. This suggests that short, low cardiovascular strain may lead to improved insulin sensitivity.

Experimental evidence of passive heat therapy is sparse, and its effects on insulin sensitivity has only recently been examined. However, the proposed mechanisms are similar to that observed in exercise trials; 1) elevated metabolic rate [Braun et al., 1995], 2) cardiac output [Faulkner et al., 2017; Rivas et al., 2016], 3) skeletal muscle blood flow [Heinonen et al., 2011], 4) skin blood flow [Crandall et al., 1999] and an elevated vasodilatory response [Brunt et al., 2016; Voulgari et al., 2013]. This suggests a potential for passive heating as an alternative to exercise to improve glycaemic control. Therefore, passive heating may offer an alternative treatment modality for individuals who cannot engage in exercise or struggle with adherence to exercise programmes [Ary et al., 1986; Barnard et al., 2000; Clark, 1997; Glasgow et al., 1997; Nathan et al., 2006; Stunkard and Messick, 1985]

3) Activation of heat shock proteins

Exercise-induced hyperthermia causes heat stress to cells all around the body [Periard et al., 2011]. Heat stress triggers the production of eHSP via mRNA inside the cells in an attempt to protect cells from damage to extreme stress [Hayden et al., 2005]. In individuals with T2DM, deleterious manifestations increase in prevalence such as chronic inflammation as physiological stress increases [Hotamisligil, 2006; Shoelson et al., 2006]. This chronic inflammation (of inflammatory cytokines such as tumour necrosis factor- alpha (TNF-α)) then leads to impaired insulin signalling by inhibiting the IκB kinase in insulin sensitive organs [Hundal et al., 2002; Shoelson et al., 2003; Yuan et al., 2001]. In tissues where impaired insulin signalling occurs, a decrease in eHSP has been seen [Atalay et al., 2004; Bruce et al., 2003, Kavanagh et al., 2009] via a reduced in concentration of heat shock factor-1 [Atalay et al., 2004; Bruce et al., 2003, Kavanagh et al., 2009]. Since eHSP is an anti-inflammatory molecule, when there are reduced levels of this protein, it causes increased inflammation [Hooper and Hooper 2009], which in turn will alter insulin function [Chung et al., 2008; Li et al., 2008]. This progressive cycle is illustrated in Figure 1.

Acute exercise results in an upregulation of HSP mRNA (coding for eHSP), which ultimately leads to protection of cells by ensuring reduced accumulation of harmful protein aggregates (phosphorylated Tau proteins and amyloid precursors) [Hayden et al., 2005]. HSP mRNA is downstream of protein expression and is indicative of future protein expression [Henstridge et al., 2010]. The advantage of using HSP mRNA over intracellular heat shock proteins (iHSP) is that it can take up to 24h for the production of iHSP from stimulation [Hunter-Lavin et al., 2004]. However, HSP mRNA presents itself almost immediately from the initiation of stimulation [Henstridge et al., 2010]. Therefore, HSP mRNA can give a more valid representation of any acute changes that may have happened from an intervention and should also mean this mechanism plays a role in reducing blood plasma [glucose] after heating as well. [Bruce et al., 2003; Chung et al., 2008; Henstridge et al., 2010 and Kurucz et al., 2002]. Surprisingly, the utility of measuring HSP70 mRNA is questionable given the transcription of the HSP70 gene (via heat shock factor) does not always result in increased protein expression [Bruce et al., 1993; Hensold et al., 1990]. It may, therefore be prudent to measure eHSP as opposed to mRNA.

Reduced eHSP in individuals with T2DM can be used as a predictor of the disease [Atalay et al., 2004, Bruce et al., 2003, Kavanagh et al., 2009]. Physiological stresses such as exercise, and passive exposure to heat cause an inflammatory response, which results in the production of eHSP [Faulkner et al., 2017].

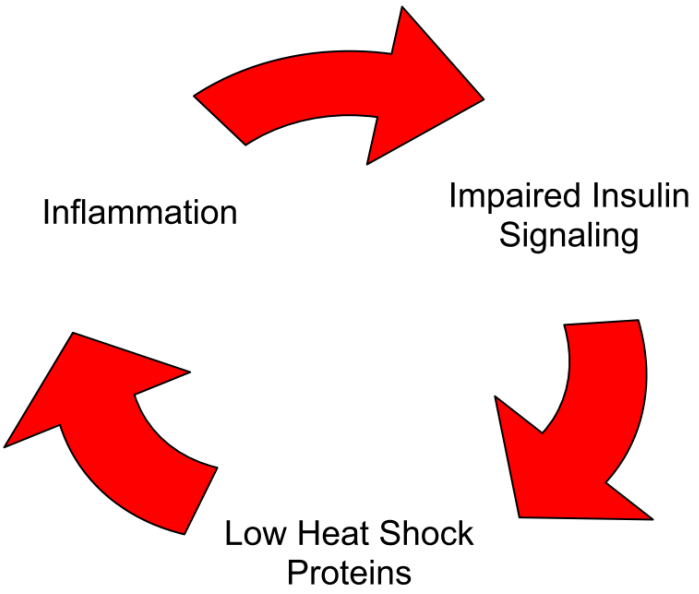


**Figure 1.** Shows the interaction of changes between inflammation, impaired insulin signalling and heat shock proteins. Taken from Hooper, 2009.

An increase in eHSP causes a reduction in inflammation by blocking the activation of inflammatory kinases and pro-inflammatory transcription factors through blocking the transcription factors targets, activation and binding [Stice and Knowlton, 2008]. A reduction in inflammatory status then increases insulin sensitivity by reducing the activation of serine-threonine kinase, c-jun amino terminal kinase and inhibitor of κ B kinase in insulin sensitive organs: liver, skeletal muscle and adipose tissue [Hotamisligil, 2006; Shoelson et al., 2006]. C-jun amino terminal kinase and inhibitor of κ B kinase both impair insulin function of the insulin receptor and interfere with downstream insulin signalling [Hotamisligil, 2006; Shoelson et al., 2006]. It is unknown whether this process is elicited through the mechanisms from exercise itself, the hyperthermia caused by exercise or a combination of both.

4) Inflammatory markers and insulin sensitivity

Reducing in inflammation may provide a means to improve insulin signalling and thus improve in glycaemic control and insulin sensitivity [Chung et al., 2008; Li et al., 2008] in individuals with T2DM. Previous studies have investigated inflammatory biomarkers in individuals with T2DM using exercise as a treatment protocol, which show that immediately after exercise there is an increase in inflammatory biomarkers, which subsequently cause an increase in anti-inflammatory biomarkers [Jorge et al., 2011; Kadoglou et al., 2007]. This data suggests that

regular exercise can result in a chronic reduction in inflammation. A potential mechanism for this may be that exercise increases metabolism and thus increases core and muscle temperature, resulting in an increased heat stress to cells in the body [Periard et al., 2011] and an increase in in eHSP and this then reduces inflammation [Chung et al., 2008; Li et al., 2008]. Faulkner et al., (2017) showed that when a healthy population underwent a passive heating protocol there was an immediate inflammatory response which they concluded would promote an anti-inflammatory response, however they also showed that 2 h post passive heating, IL-6 inflammatory response reduced to almost baseline levels again. In contrast, any initial increase in inflammatory biomarkers can be used as an indicator for a longer term increase in anti-inflammatory biomarkers [Steensberg et al., 2003]. Steenburgs et al., (2003) results showed that there was a delayed anti-inflammatory response ([IL-1ra] and [IL-10]) of 60 min from infusion of IL-6.

To date, evidence of passive heating on anti-inflammatory biomarkers is scarce in individuals with T2DM. To date, one study has shown found that after a 6-month exercise training programme there was a reduction in inflammatory biomarkers (TNF-α, high sensitivity CRP, IL-18) and an increase in resting anti-inflammatory biomarker (IL-10) concentrations (Kadoglou et al., [2007]). Therefore, more data on the anti-inflammatory response to passive heating in individuals with T2DM is needed.

5) Autonomic function

Individuals with T2DM have reduced autonomic function which leads to, reduced cardiac and vascular function, and cardiovascular disease [Voulgari et al., 2013] which is caused at least in part by chronic hyperglycaemia [Kitsios et al., 2011; Papanas et al., 2011]. Chronic hyperglycaemia induces several metabolic pathways to undergo a repetitive vicious cycle which leads to micro- and macrovascular damage [Kitsios et al., 2011; Papanas et al., 2011]. Hot environments may, via the same physiological outcomes as exercise, cause cardiovascular strain and so may be a long term way of training the heart, without needing to exercise. This may improve the cardiovascular outcome for individuals with T2DM [Faulkner et al., 2017].

Simple, inexpensive interventions aimed at improving glycaemic control in individuals with T2DM are needed to help the growing health and economic burden. Using passive heating as a therapeutic strategy for maintaining glucose homeostasis, and improving metabolic and cardiovascular health is a novel, developing field, worthy of further investigation. Considering the above, there is a clear rationale to explore further whether passive heating has therapeutic potential in individuals with T2DM. We therefore propose to investigate the effect of passive heating on glucose tolerance (plasma [glucose]), plasma [insulin], insulin sensitivity, interleukin 6 concentration [IL-6], [IL-10], [eHSP-70], HRV, muscle blood flow and thermal sensation / comfort.

## 5. PRELIMINARY STUDIES AND EXPERIENCE OF INVESTIGATORS

Dr. Anthony Shepherd is a Senior Lecturer in Physical Activity, Exercise and Health at the University of Portsmouth. During his career he has worked with several clinical populations, including T2DM, stroke, geriatrics, Raynaud's and scleroderma, cystic fibrosis (CF), end-stage renal disease and chronic obstructive pulmonary disease. This work was undertaken at the University of Exeter Medical School, the Royal Devon and Exeter NHS Foundation Trust Hospital, South Devon Healthcare NHS Foundation Trust, Portsmouth Hospitals Trust and University Hospital Southampton where he held honorary contracts. His research has an overarching theme of clinical exercise physiology and therapeutic treatments. His most recent publications have looked at the effect of increasing the bioavailability of nitric oxide ($NO^{\bullet}$) in clinical cohorts via nutritional supplementation. He has disseminated this work in internationally recognised journals and holds research grants from charities and industry partners.

Mr. Thomas James completed his BSc at the University of Roehampton in Sport and Exercise Science. During his time there he became interested in physiology in extreme environments and conducted his final year project investigating the cardiovascular and thermoregulatory adaptations of a high-intensity interval training, short-term heat acclimation protocol in trained cyclists. He then attended Bangor University and completed an MRes. in Sport and Exercise Physiology. During his time there he focused his research more on cardiovascular physiology in extreme environments. His research and thesis for his MRes. investigated the autonomic control of cardiovascular responses to the human diving reflex. For Thomas' PhD research, he will be applying this knowledge and skill base to drive research into novel and inexpensive T2DM treatments. More specifically, looking at the metabolic and cardiovascular health of individuals with T2DM while using extreme stressors (e.g. warm water) with the aim of developing a new, inexpensive and adherable treatment. The research has the potential to help reduce the economic burden on the NHS, as well as improving the well-being of individuals with T2DM. Mr James will be assisted by two postgraduate students, Mr Billy Hopkins and Mr Connor Morgan. A third year student, Miss Emily Windsor will also assist in data collection. All students have valid GCP certificates and have been training in all procedures.

Dr. Jo Corbett has worked at the University of Portsmouth since 2005 and is currently Associate Head for Innovation and Impact in the Department of Sport and Exercise Science as well as the REF coordinator for UoA24. His PhD was awarded by the University of Southampton and examined pharmacological and non-pharmacological approaches to increasing fat metabolism. Subsequently his research has focused on environmental stressors. He is an established research leader in sport, exercise and occupational activities in hot environments with 50+ peer-reviewed publications in the field; he was lead author on the 2018 British Association of Sport and Exercise Sciences Expert Statement on Interventions for Improving Performance in the Heat. Jo is an advisor to the English Institute of Sport on exercise in the heat and is a British Association of Sport and Exercise Sciences accredited Physiologist.

Dr. Joseph Costello is a Senior Lecturer in Exercise Physiology at the University of Portsmouth. He completed his PhD in exercise physiology at the University of Limerick, Ireland. After his PhD, he moved to Queensland University of Technology (Australia) where he worked as a post-doctoral research fellow in the Institute of Health and Biomedical Innovation for three years. Joseph then undertook further post-doctoral research in the Extreme Environments Laboratory with the Department of Sport and Exercise Science at the University of Portsmouth, before being appointed as a Lecturer in 2016 and recently Senior Lecturer. Dr. Costello is currently on the editorial board of Experimental Physiology, the commissioning editor for Extreme Physiology & Medicine, and a methodological editor for the Cochrane Bone, Joint and Muscle Trauma Group.

Prof. Mike Tipton joined the University of Portsmouth from University of Surrey in 1999. In addition to his University positions, he was based at the Institute of Naval Medicine from 1983 to 2004 and was Consultant Head of the Environmental Medicine Division from 1996. Mike has spent 30 years working in the areas of thermoregulation, environmental and occupational physiology. Mike and is colleagues in the Extreme Environments Laboratory examine the physiological and psychological responses to adverse environments and the selection, preparation and protection of those who enter such environments. He is Director of Research for the Department of Sport & Exercise Science, he provides advice to a range of universities, government departments, industries, medical, search and rescue and media organisations.

Prof. Janis Shute is a Professor of Pharmacology at the University of Portsmouth and is head of the Respiratory Immunopharmacology group, investigating mechanisms and biomarkers of lung inflammation and novel strategies to improve inhaled drug delivery in patients. Prof. Janis Shute has worked to develop novel inhibitors of neutrophil elastase, a key mediator of tissue damage in the lung. Other research includes the role of coagulation factors in bronchial epithelial damage and repair in normal and asthmatic airways; endothelial dysfunction and the links between inflammatory markers and oxidative stress markers. She will advise on biochemical analysis, processing of samples, storage of samples and aid with protocol and manuscript development.

Prof. Michael Cummings is a Consultant in Diabetes and Endocrinology at the Portsmouth Hospitals Trust and is also an Honorary Professor in Diabetes and Endocrinology at the University of Portsmouth. His major areas of interest in diabetes are linked to cardiovascular disease and risks (in particular dyslipidaemia and microalbuminuria), diabetes and pregnancy and erectile dysfunction. His primary research interests are in endothelial dysfunction, oxidative stress and dysglycaemia. He secured funding and established a small laboratory to undertake non-invasive digital plethysmography and venous occlusion plethysmography, techniques used to examine endothelial function. This technique has been applied to examine the mechanisms of endothelial dysfunction in type 1 and T2DM. He also collaborated with the University of Portsmouth to develop the DOVE (Dysglycaemia, Oxidative stress and the Vascular Endothelium) project and laboratory, developing assay techniques to measure markers of oxidative stress and vascular inflammation. Additionally, he has collaborated with our team in the Department of Sport and Exercise Science on other projects. Many oral presentations/abstracts/original papers based upon this work have been

presented at national/international meetings and the findings have been accepted in peer-reviewed journals for publication. He will bring an expert clinical opinion to help design and inform the protocol.

Dr Iain Cranston is a Consultant in Diabetes and Endocrinology at the Portsmouth Hospitals Trust. He is also the Clinical Lead for Diabetes and Intensified Insulin Therapy, Diabetes inpatient care and Diabetes and Renal Disease. Iain is an expert in continuous glucose monitoring (CGM) and diabetes, and will help with protocol design and data analysis. He will bring an expert clinical opinion to help inform the protocol and will help with participant recruitment.

Dr. Zoe Saynor is a Senior Lecturer in Physical Activity, Exercise and Health at the University of Portsmouth, whilst also holding several visiting academic positions at University College London and the University of Toulon. She has 10 years' experience conducting research and applied work with individuals with various chronic health conditions, including CF, asthma, non-CF bronchiectasis, rheumatoid arthritis, renal disease, diabetes (T2DM and CF-related), Raynaud's phenomenon and joint hypermobility. She has particular expertise in CF, having published numerous research articles and delivered several invited talks on the topic, however she has a varied clinical portfolio of research at present. Since her time at the University of Portsmouth, she has embedded clinical research within both the University Hospital Southampton and Queen Alexandra Hospital (Portsmouth) and has established the Clinical, Health and Rehabilitation research Team within the Department of Sport and Exercise Science at the University of Portsmouth.

## 6. AIMS AND OBJECTIVES

The global aim of this investigation is to establish if an acute bout of passive, warm water therapy reduce plasma [glucose] in the blood in response to an OGTT.

### 6.1 Primary Objective

1. To determine if an acute bout of passive, warm water therapy reduces plasma [glucose].

### 6.2 Secondary Objectives

*To determine:*

1. whether plasma [glucose] reduces more if the passive, warm water therapy is conducted before or after the OGTT?
2. whether plasma [insulin] reduces more if the passive, warm water therapy is conducted before or after the OGTT?
3. whether insulin sensitivity increases following an acute bout of warm water therapy?
4. whether fuel utilisation alters during and following an acute bout of warm water therapy?
5. whether cardiovascular status is altered during or after an acute bout of warm water therapy?

6. whether eHSP increases during an acute bout of warm water therapy?
7. whether inflammatory status reduces during or after an acute bout of warm water therapy?

### 6.3 Summary of Study Design

Recruitment

Participants will be recruited from adult diabetes outpatient clinics at the Queen Alexandra Hospital (Portsmouth), via a database of known individuals with T2DM from the Wessex, Diabetes, National Institute for Health Research (NIHR) Clinical Research Network and via posters and word of mouth at the University of Portsmouth. Only males and post-menopausal women will qualify for the study. Finally, the local Desmond diabetes awareness group meetings will be used to identify potential volunteers. Potential participants will either be approached by their T2DM consultant, the principal investigator, member of the research team or a research nurse. Following the participants declaration of interest, via telephone, mail, email or in clinic, they will be given a participant information sheet. A member of the research team will subsequently approach the participant ≥ 48 h later, to answer any questions and organise consent, screening and familiarisation (visit 1).

The design will be a balanced, randomised, within-subject comparison of three conditions (condition 1: OGTT in 23 ºC air, condition 2: OGTT 30 min before 60 min in ~39ºC water (~ 90% RH), condition 3: OGTT 30 min after 60 min in ~39°C water (~ 90% RH)). There will be 4 visits in total, visit 1 is for consent, screening and familiarisation and visits 2, 3 and 4 are experimental conditions.

Visit 1 (consent, screening and familiarisation)

During visit 1, participants will give their informed consent, followed by a health screening questionnaire. In addition to the health screening questionnaire, medical history and a blood sample will be collected and analysed for a full blood count, glycated haemoglobin ($HbA1_c$), liver and kidney function. Finally, a resting electrocardiogram (ECG) will also be recorded and then examined for irregularities, where a clinical decision will be made on further participation to the study by consultants at QA hospital. Participants will then be shown the rest of the equipment and taken through the procedure for the next 3 visits and, if the participant is happy to continue the study, the next visit will be organised.

*Pre experimental instructions*

Before all experimental visits the participants will be instructed to keep their normal diet up to 48 h before coming in for their visit and also replicate it before each visit (they will be given a food diary). They will be asked to avoid exercise and hot baths / saunas 48 h before each visit (can have brief (< 5 minutes) luke-warm showers) and consume 17 $mL.kg^{-1}$ water (for examples an 80 kg male would need to drink at least 1.36L of water) and other fluids ad libitum throughout the active day (~ 16 h) [Benelam and Wyness, 2010] and 4 $mL.kg^{-1}$ upon waking the morning of the visit. All conditions will require the participant to fast for 12 h before coming

to the start of the visit. For the pre-test diet, participant will be asked to keep a normal balanced diet (they will record their intake in a diary). Participants will also be instructed on suitable clothing to wear for each visit (explained again in the PIS). In line with OGTT guidelines, participants will be asked to not take their diabetes drugs on the morning of the test (hypoglycaemic agents before the fasting period are to be taken as normal). Prior to leaving the laboratory, participants will be provided with lunch, then asked to take their tablets and blood sugars checked prior to leaving.

Visit 2, 3 and 4

Participants will arrive at the laboratory at ~9 am for conditions 1 and 2 and 8 am for condition 3. Prior to a 15 min resting period (supine) before any measures are taken participants will be asked to insert a rectal thermistor (participants will be given clear instructions using our SoPs). Condition 1, 2 and 3 will be balanced and participants randomly allocated to begin the study in either visit 2, 3 or 4 using a blinded member of the team.

For all visits (see figure 2), participants will lie in a semi recumbent position in minimal clothing (bathing shorts and a t-shirt) for the entirety of the visit. Initially, participants will be cannulated (Versatus winged and ported IV cannula, Terumo, Japan) and blood samples drawn for analysis of osmolality (Lithium Heparin (LH) tubes BD, USA) plasma [glucose] (Fluoride/Oxalate tubes, BD, USA), [insulin] (Ethylenediaminetetraacetic acid (EDTA) K2, BD, USA), and [eHSP70] (EDTA K2, BD, USA) at baseline and every 30 min of each experimental visit. Following cannulation an 180 min OGTT (75g) (Rapilose OGTT solution, Penlan healthcare, Japan) will commence in a thermoneutral room (~ 23$^{o}$C). A maximum of 18 mL of blood being drawn at each time point (max 126 mL per visit). To maintain the patency of the cannula and to reduce the risk of infection, after every sample is taken, 5 mL of saline will be flushed through the cannula. Then before every sample is taken, 2.5 mL of blood will be drawn out of the cannula to ensure any remaining saline will not interfere with the samples and data interpretation (additional 17.5mL per visit). During the OGTT, HR (via electrocardiogram) will be measured continuously, whilst blood pressure (M5-1, Omron, Japan), deep body temperature (rectal probe) and resting metabolic rate (indirect calorimetry) (Quark CPET, Cosmed, Italy) will be assessed every 30 min.

Condition 2 will employ identical procedures to condition 1, except thirty minutes into the OGTT, the participant will be immersed into an immersion tank (~39$^{o}$C) for 60 min. Water temperature will be manipulated as required to achieve and maintain a target $T_{rec}$ at 38.5 $^{o}$C using water between 37.5 and 39$^{o}$C, and then participants will be removed horizontally back into the thermoneutral room for the reminder of the OGTT. Participants will be towel dried and given a towelled robe to wear. Condition 3 will employ identical procedures to condition 2, with the exception that the heating via immersion will start as soon as the participant is instrumented (and following a 15 min rest period) and the OGTT will commence 30 min after the 60 min immersion time for a further 180 min (see figure 2 for a schematic). Finally, if willing participants will be asked to take part in a brief exit interview about their experience.

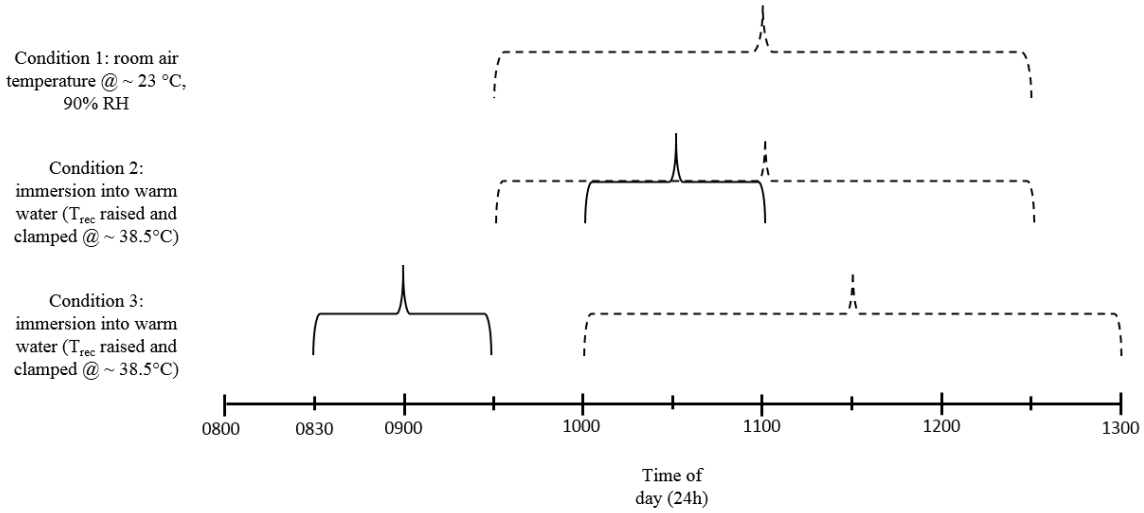


**Figure 2.** Schematic of the 3 experimental conditions. BP, thermal sensation, HRV, osmolality, insulin sensitivity, RMR, eHSP70, IL-6, IL-10, plasma glucose and insulin measured at 30 min intervals from 0930-1230 for visit 1 and 2, and from 1000-1300 for condition 3 (with the addition of a baseline measure at 0830 for visit 3). Muscle oxygenation at 0930, 1000, 1130, 1200 and 1230 for condition 1 and 2. Muscle oxygenation at 0830 and from 1000-1300 at 30 min intervals for condition 3. HR continuous for entirety of visit. Solid line indicates immersion period and dashed line indicates OGTT period.

### 6.4 Primary and Secondary Endpoints/Outcome Measures

1. To determine if an acute bout of passive, warm water therapy reduces plasma [glucose].

Secondary outcomes are to determine:

1. whether plasma [glucose] reduces more if the passive, warm water therapy is conducted before or after the OGTT?
2. whether plasma [insulin] reduces more if the passive, warm water therapy is conducted before or after the OGTT?
3. whether insulin sensitivity increases following an acute bout of warm water therapy?
4. whether fuel utilisation alters during and following an acute bout of warm water therapy?
5. whether HRV increases during or after an acute bout of warm water therapy?
6. whether eHSP increases during an acute bout of warm water therapy?
7. whether inflammatory status reduces during or after an acute bout of warm water therapy?

# 7. STUDY PARTICIPANTS

## 7.1 Study Setting

Consent, screening and familiarisation will commence at the diabetes centre and Queen Alexandra hospital. All experimental visits will take place in the Extreme Environments Laboratory at the University of Portsmouth has a 30-year history of conducting innovative human research trials. The laboratory contains world leading facilities for conducting environmental research including three bespoke environmental chambers, with temperature, humidity, simulated solar, wind and hypoxia control, and a research group involving over 20 academics and researchers.

## 7.2 Overall Description of Study Participants

Adults with a clinical diagnosis of T2DM, as defined by the WHO. A standard medical history and clinical examination will be undertaken by a research nurse or a member of the research team. Information taken during the examination will be height, weight, full blood count (analysed from a blood sample that will be collected), HbA1c, neuropathy severity and a resting ECG. ECG will be examined for irregularities and a clinical decision will be made on further participation to the study by a consultant at QA hospital.

## 7.3 Eligibility Criteria

**Inclusion Criteria**

The participants must meet ALL of the following criteria to be considered eligible for the study:

- Male or female (either post-menopausal or in the early-follicular phase (3-5 days after the onset of menstruation) of the menstrual cycle), aged 35 years or above.
- Diagnosed with T2DM as defined by the WHO.
- Participant is willing and able to give informed consent for participation in the study.
- Participant is able to understand and fully cooperate with the study protocol.

Exclusion Criteria

The participant may not enter / be withdrawn from the study if ANY of the following apply:

- Severe peripheral neuropathy (to the point to which they cannot sense temperature)
- Uncontrolled hypertension (≥180 systolic / 100 diastolic mmHg)
- Taking any medication which may interfere with data interpretation or safety
- Who have had a myocardial infarction or cerebro−vascular event
- Any cardiac abnormalities which restrict hard exercise
- Current smokers or who have stopped within 3 months
- Participant is unable to understand and/or fully cooperate with the study protocol

- Any other serious medical condition which would interfere with data interpretation or safety will be excluded from participation.
- Any skin conditions including ulcerations

## 8. SAMPLING

Plasma glucose AUC is our primary outcome. No study to date has assessed the effect of hot water immersion on glucose tolerance in individuals with T2DM. Therefore, no data were available to power our primary outcome. For 90% power with an α-level set at $P < 0.05$ (two tailed), to detect a 1 SD difference, 13 volunteers are required to compare within group for placebo and active conditions. In order to counterbalance our design, 18 participants will be required to complete the testing. Therefore, we aimed to recruit 20 individuals with T2DM to account for a 10% drop-out rate typically seen in our clinical trials. Resultant effect sizes will be used to power future trials.

## 9. STUDY PROCEDURES

### 9.1 Recruitment

Participants will be recruited from adult diabetes outpatient clinics at the Queen Alexandra Hospital (Portsmouth), via a database of known individuals with T2DM from the Wessex, Diabetes, National Institute for Health Research (NIHR) Clinical Research Network and via posters and word of mouth at the University of Portsmouth. Additionally, participants will be recruited through primary care and via posters. Finally, the local Desmond diabetes awareness group meetings will be used to identify potential volunteers. Potential participants will either be approached by their T2DM consultant, the principal investigator, member of the research team or a research nurse. Following the participants declaration of interest, via telephone, mail, email or in clinic, they will be given a participant information sheet. A member of the research team will subsequently approach the participant ≥ 48 h later, to answer any questions and organise consent, screening and familiarisation (visit 1). A maximum payment of £10 will be available per visit upon request.

### 9.2 Screening and Enrolment

Consent and screening will occur at the diabetes centre at Queen Alexandra hospital. A standard medical history and clinical examination will be undertaken by a research nurse or a member of the research team at the screening visit (visit 1) after consent has been taken, including height (stadiometer, Seca, Germany), weight (Ohaus, USA), ECG and blood sample collection for lipids and biochemical markers, urea and electrolytes, HbA1c and liver function tests.

Blood pressure: Semi recumbent resting blood pressure: measurements will be performed on the morning of the screening visit; serial measurements on each participant will be made at the same time of day and same arm for each visit. The recordings will be made in quiet, comfortable, ambient laboratory conditions. Measurements will be made with a semi−automated device conforming strictly to European Hypertension Society (as adopted by

the British Hypertension Society) and American Heart Association guidelines. Five measurements will be made and the final three will used to determine an average blood pressure.

### 9.3 Randomisation

An online computer programme will be used to randomly allocate and counterbalance participants to being the trial in one of three conditions. This will be performed by a blinded member of the research team.

### 9.4 Study Assessments

*Pre-trial instructions*

Participants will be instructed to keep their normal diet and to keep it the same 48 h before each trial (they will record their intake in a diary). Additionally, they will have to fast for 12 h before the start of each visit. Participants will be instructed to consume 17 $mL.kg^{-1}$ of water and other fluids ad libitum throughout the active day (~16 h) the day before each visit [Benelam and Wyness, 2010] and 300 mL upon waking the morning of the visit. Participants will also be told to bring a bathing suit and a t-shirt to wear for the experimental visits. Additionally, participants will also be told that they cannot perform exercise or have a hot bath / sauna 48 h before each visit (can have brief (< 5 min) luke-warm showers). Visits to the laboratory should be made either via car or public transport and should not be made via physical activity e.g. cycling. Parking will be provided. At the start of each visit, plasma osmolality will be assessed via freezing point depression (model 3320 osmometer, advance instruments, USA). In line with OGTT guidelines, participants will be asked to not take their diabetes drugs on the morning of the test (hypoglycaemic agents before the fasting period are to be taken as normal). Prior to leaving the laboratory, participants will be provided with lunch, then asked to take their tablets and blood sugars checked prior to leaving.

*Passive heating and Core Temp*

The passive heating conditions (conditions 2 and 3) will be conducted in a thermoneutral room (~ 23°C air, ~ 90% RH), at the same time of day, with the heating taking place for 60 min in a heated pool (starting at 39°C water). Pre and post body mass will also be recorded, along with any fluid or solids going into or out of the body. Participants will firstly be cannulated lying horizontal, then will lie semi-recumbent and baseline measures taken. Depending on which condition (either 2 or 3) the OGTT or the passive heating will start first. Passive heating will start with the participant entering the pool and lying semi-recumbent on a submerged bed with their head, neck and cannulated arm above the water. Participants' rectal temperature ($T_{rec}$) will be monitored so that once it reaches ~38°C the water will be cooled to ~ 38 - 38.5°C so that it will maintain $T_{rec}$ at ~ 38.5°C ($T_{rec}$ is still expected to rise slightly after reduction in water temperature, see below for more details). If $T_{rec}$ reaches 39°C or above at any point, the participant will immediately be withdrawn from the study and a cooling protocol administered (see SOP attached). Additionally, if systolic BP raises above 180 mmHg or HR above 140

bpm, participants will be immediately withdrawn from the study. Passive heating will cease by winching the participant, still lying semi-recumbent, out of the immersion tank and onto a semi-recumbent bed. The participant will then be towel dried and given a blanket (to ensure maximum heat retention) and remain semi-recumbent until completion of the visit. The reminder of the visit will be conducted in the thermoneutral room.

$T_{rec}$ will be measured continuously throughout each visit using disposable rectal thermistors (Rectal temperature probe, Philips, Netherlands). Participants will self-insert the thermistor to 15 cm (this will be marked with sterilised tape) into the rectum using standard operating procedures. $T_{rec}$ will be closely monitored throughout the passive heating conditions to ensure that it does not exceed 39°C.

*OGTT and Insulin Sensitivity*

Insulin sensitivity will be calculated using values from both plasma glucose and plasma insulin. Multiple calculations for insulin sensitivity exist, each with their own strengths and weaknesses. Calculations used can be split into two categories: fasting indices and OGTT indices. A meta-analysis [Otten et al., 2014] of all the current surrogate measures of insulin sensitivity showed that the most valid fasting calculation to use compared with the gold standard HEC method, was the quantitative insulin sensitivity check index (QUICKI). Out of the OGTT surrogate insulin sensitivity measures Otten et al. (2014) highlighted 4 indices that were the most suitable surrogate for HEC: Stumvoll, OGIS, Matsuda and Gutt.

Following an overnight fast, blood samples will be taken for the measurement of fasting glucose and insulin levels, and this data will be used to calculate β-cell function via the QUICKI model [Katz et al., 2000], $1/[\text{Log}(I_0)+\text{Log}(G_0)]$ (I=insulin, G=glucose, $_0$=baseline) which has been shown to have a strong positive correlation with the HEC [Otten et al.,2014]. Post-prandial glycaemic control will be measured by a 180 min OGTT (maximum of 75 g anhydrous glucose), with blood samples taken from a cannula at 0, 30, 60, 90, 120, 150 and 180 min of the OGTT, with β-cell function being calculated using the Stumvoll method (Equation 1).

$$0.226-0.0032\text{xBMI}-0.0000645\text{x}I_{120}-0.00375\text{x}G_{90}$$

**Equation 1.** Stumvoll's insulin sensitivity index equation [Stumvoll et al., 2000]

*Thermal sensation / comfort*

Both thermal comfort and sensation will be measured using a 20 cm scale (0 = very cold/uncomfortable; 10 = neutral; 20 = very hot/comfortable; modified from Zhang et al., (2004) and recorded prior to immersion, during immersion and following the 60 min immersion, at 15 mins intervals.

*Resting Metabolic Rate (metabolite utilization)*

Breath-by-breath changes in pulmonary gas exchange and ventilation will be non-invasively measured at rest to enable the estimation of resting metabolic rate (RMR). The assessment of pulmonary gas exchange (e.g. $\dot{V}O_2$ and $\dot{V}CO_2$) will give us the respiratory exchange ratio (RER). RER is a reliable and valid measure of fuel utilisation. To assess RMR, participants will be asked to lie down and rest for 15 mins, following this, pulmonary gas exchange will be measured via a gas analysis system for 5 min, this will be repeated every 30 min until the end of the visit. Participants will be required to use a mouth piece with a nose clip. In order to assess inspired and expired gas (volume and concentration), gases will be sampled through a capillary line attached to the face mask. A sub analysis of this breath by breath data will give us the respiratory exchange ratio (a value of 0.7 indicates only fatty acid metabolism and values of > 1 indicates only glucose metabolism, any value in the middle equates to a combination of both). This is an accurate assessment of rate of energy expenditure and fuel utilisation, and will be indicative of what proportion of carbohydrate and fat is being metabolised [Blond et al., 2011]. In order to calculate the rate of carbohydrate and fat metabolism the Frayn (1983) equations will be used. Pulmonary gas analysis will be performed on all testing visits. Calibration will follow the guidelines given in the user manual produced by COSMED Omnia. Briefly, this involves calibrating the gas analyser by passing through a sample of gas with a known concentration (Oxygen: 16%, Carbon dioxide: 5%, Nitrogen: Bal) and calibrating the volume on the gas flowmeter by using a 3 L syringe to pass a known volume of air through the flowmeter.

*Near- infrared spectroscopy (NIRS)*

Muscle oxygenation in the microcirculation of the *m. vastus lateralis* will be measured using a commercially available NIRS system (Portamon, Artinis, Netherlands) during the pre- and post-immersion periods. This system is non-invasive and will be placed on *m. vastus lateralis* of the right leg. NIRS is used to estimate changes, relative to baseline, in oxygenated and deoxygenated haemoglobin concentrations, myoglobin concentrations, total haemoglobin and tissue saturation index to give an insight into the dynamic balance between muscle $O_2$ delivery and utilisation. Furthermore, a skin-fold measurement at the site in which the NIRS device is fixed will be recorded, in line with the manufacturer's recommendations.

*HR and BP*

HR will be automatically calculated from the ECG, real-time. Blood pressure will be measured every 30 min via a patient monitor brachial BP cuff (Dynascope, Fukuda Denshi, Japan).

*HRV*

HRV will be calculated as a low frequency (LF) variable and root mean square of successive differences (RMSSD). LF has been chosen as it indicates a physiological origin of both sympathetic and vagal activity. RMSSD is then being calculated additionally as it gives a stronger representation of vagal tone (due to it being affected much less by respiratory sinus

arrhythmia (RSA)) than LF thus improving validity of the vagal tone measure [Laborde et al., 2017]. Both measures will be calculated from an ECG trace.

*Biochemistry*

Venous blood samples will be collected via cannulas (ideally located in the Cephalic vein on the forearm, alternatively ether in hand or antecubital fossa. All locations will out of the water at all times) into fluoride, LH and EDTA vacutainers during all visits. All whole blood will be centrifuged at 4500 x g for 10 mins, at 4 °C and aliquoted plasma will be frozen at -80 °C for subsequent analysis.

Plasma [glucose] will be determined by the Department of Biochemistry at the Queens Alexandra Hospital, Portsmouth. Commercially available enzyme-linked immunosorbent assays will be used to determine [insulin], [IL-6], [IL-10] and [eHSP70]. Haemogloin and haematocrit will be measured via Haemacue (Hb 201, Sweden) and capillary tubes (Microhaematocrit capillary tubes, Hawksley, UK) respectively at all data collection time points in triplicate.

*Follow-up questions*

A few semi-structure follow-up questions will be given to the participants to get feedback on how they felt in the hot water in both conditions. The questions will be related to how they felt in terms of heat tolerance, lethargy, well-being and also how they felt in the hours and days following each visit.

### 9.5 Discontinuation/Withdrawal of Participants from Study Treatment

Participants may be withdrawn from the study if they ask to withdraw, if participant safety is in question or if the study is stopped early.

### 9.6 Definition of End of Study

The end of study will be following the final dissemination of results, which is expected to be during September 2019.

## 10. INTERVENTIONS

### 10.1 Description of Study Intervention / Treatment

The passive heating condition will be conducted in a thermoneutral room (~ 23°C air, ~ 90% RH) with the heating taking place for 60 min in a heated pool (starting at 39°C water (~ 90% RH) and then cooling to clamp $T_{rec}$ at 38.5°C). The participant will firstly be cannulated, lie horizontal and baseline measures taken after 15 min rest period. Following baseline measures an OGTT will start. Thirty min into the OGTT, the passive heating will start with the participant entering the pool and lying horizontally on a submerged bed with their head and neck elevated

above the water. Participants' rectal temperature ($T_{rec}$) will be monitored so that once it reaches 38°C the water will be cooled slightly to arrest the rise in $T_{rec}$ and maintain $T_{rec}$ at 38.5°C ($T_{rec}$ is still expected to rise slightly after reduction in water temperature, see below for more details). If $T_{rec}$ at any point reaches 39°C or above, the participant will immediately be withdrawn from the study and a cooling protocol administered (see SOP attached). Passive heating will cease by winching the participant out of the pool and onto the dry bed with the participant still lying in a semi-recumbent position. The participant will then be dried and given a blanket (to ensure heat retention as would be the case with putting on clothing after bathing) and remain semi-recumbent until completion of the visit. The reminder of the OGTT will be conducted in the thermoneutral room (~23°C room temperature).

Core temperature ($T_{rec}$) will be measured continuously throughout each visit using disposable rectal thermistors (which will be checked for accuracy in a water bath prior to each trial ± 0.2°C). Participants will insert the thermistor 15 cm (this will be marked with sterilised tape) into the rectum using standard operating procedures. $T_{rec}$ will be closely monitored throughout the passive heating condition to ensure that it does not exceed 39°C. For analysis, data will be grouped into 1 min average intervals (and will be collected at a 1 Hz sampling frequency).

### 10.2 Adherence to Study Treatment

Participants will be immersed into the pool and core temperature measured to ensure safety. Participants can, at any time, ask to be safely removed from the pool. Additionally, participant will be removed from the pool if $T_{rec}$ reaches >39°C. Total time in the pool will be calculated for each participant on each visit.

### 10.3 Accountability of the Study Treatment

**Any of the study investigators are able to be accountable for maintaining the appropriate water temperature.**

### 10.4 Concomitant Medication / Therapies

Participant involvement is anticipated to be 3 morning sessions within a period of ~3 weeks. Medication will be recorded on enrolment and updated throughout their time on the trial. For each experimental visit, hypoglycaemic aids will be stopped for the morning (see pre experimental instructions for details).

## 11. ASSESSMENT OF SAFETY

### 11.1 Definitions

A serious adverse event (SAE) is any untoward medical occurrence that:

- Results in death
- Is life-threatening*

*The term "life-threatening" refers to an event in which the participant was at risk of death; it does not refer to an event which hypothetically might have caused death if it were more severe.

- Requires inpatient hospitalisation or prolongation of existing hospitalisation
- Results in persistent or significant disability/incapacity
- Is a congenital anomaly/birth defect
- Other important medical events*

*Other events that may not result in death, are not life threatening or do not require hospitalisation may be considered a SAE when, based upon appropriate medical judgement, the event may jeopardise the participant and may require medical or surgical intervention to prevent one of the outcomes listed above.

### 11.2 Reporting Procedures for Serious Adverse Events

SAE occurring to participant should be reported to the research ethics committee (REC) that gave a favourable opinion of the study where in the opinion of the Chief Investigator the event was: 'related' – that is, it resulted from administration of any of the research procedures; and 'unexpected' – that is, the type of event is not listed in the protocol as an expected occurrence. Reports of related and unexpected SAEs should be submitted within 15 days of the Chief Investigator becoming aware of the event, using the national research ethics service (NRES) report of serious adverse event form (see NRES website).

### 11.3 Recording and Reporting Procedures for All Adverse Events

All adverse events will be recorded in participant file notes. Distinction between SAE and adverse event (AE) will be made by an independent medical officer. All SAE will be reported to the sponsor and the REC regardless of if it is related or not.

## 12. DATA HANDLING AND RECORD KEEPING

### 12.1 Data Collection Forms

Visit 1 will act as a screening and consent (ECG checked by clinical physician and blood to be analysed for a full blood count, $HbA1_c$, estimated glomerular filtration rate (eGFR) and liver function). All measures for this visit will be stored in the clinical records folder (CRF). Separate forms will be prepared for; anthropometrics, medical history, screening (i.e. blood pressures etc) and blood markers. On subsequent visits data forms will be used to collect temperatures of the labs and immersion pool. All paper copies will be stored in the CRF's.

### 12.2 Data Management

All data will be double data entered into excel. Macro's will be used to check for anomalies and corrected. The participants will be identified by a study specific participants number and/or code in any database. The name and any other identifying detail will not be included in any

study data electronic file. The Chief and Principal Investigators are responsible for database maintenance and management.

## 13. DATA ANALYSIS

### 13.1 Description of Analysis Populations

Participants who completed all 4 visits will be entered into the analysis.

### 13.2 Analysis of Endpoints

All data will be tested for normality. Normally distributed data will be presented as means ± SD. Statistical analyses will be performed on SPSS software version 24.0 (Chicago, IL).For normally distributed data a one-way analysis of variance (ANOVA) will be used to identify differences between conditions for plasma [glucose] (AUC and peak), plasma [insulin], insulin sensitivity, [eHSP70], [IL-6], [IL-10], HRV, NIRS and RMR. Where significance differences are observed, post hoc test will then be performed. Where data is not normally distributed, appropriate non parametric tests will be utilised.

### 13.3 Procedure for Dealing with Missing, Unused and Spurious Data

Outliers will remain within the data. Within the data analysis software missing data will be coded 9999 and the data point will be missed during analysis.

### 13.4 Procedures for Reporting any Deviation(s) from the Original Statistical Analysis Plan

For this study a statistical analysis plan is not required (excluding the above).

### 13.5 Interim analysis and criteria for early study termination

Due to the small samples size and time frame, no interim analysis will be performed. Early termination will only occur if the safety of participants is in question.

## 14. ETHICS

NHS Local Research Ethics Committee (REC) approval will be obtained prior to commencement of the study. The REC, local NHS, research and development department and all site specific forms and patient identification centre forms will be forwarded to the R&D department at Queen Alexandra Hospital Portsmouth, prior to recruitment of participants. Written informed consent will be obtained from all participants. Insurance indemnity will be provided by the University of Portsmouth for this study.

Every effort has been made to keep the risks and discomforts to a minimum, with first aid cover and extensive screening procedures in place but there are some risks associated to taking part. Not all volunteers will experience any or all of the risks stated below, but participants will be made aware of them.

The main burden to participants is a 12 h fasting period before their visit. Participants will be asked to cease all hypoglycaemic agents on the morning of testing and will be instructed to bring them with them on the day. This is in line with a normal fasted OGTT typically used in clinics. Prior to leaving the laboratory, participants will be provided with lunch, they can take their tablets and blood sugars checked prior to leaving.

We will also be asking people with T2DM to be submerged in hot water for condition 2 and 3. This may cause some discomfort which will be assessed with a thermal sensation and comfort scale. We will be measuring the core temperature via rectal thermistor and monitoring it closely at all times. Participants will be reminded that they are free to withdraw at any time point and will also be withdrawn and cooled if $T_{rec}$ raises above 39°C (as stated in the departments SAP's).

Participants will be asked to insert a rectal thermistor on two out of the four visits (water immersions). This can be uncomfortable upon insertion; however, this is necessary for safety purposes. Participants will be provided with lubrication and instructions.

Other less burdensome tests will be performed such as:

Blood pressure; participants will feel a cuff squeeze their arm and this can be uncomfortable for a few moments. Blood samples taken every 30min via cannulation (using the appropriate hygienic technique and by a trained individual): Intravenous access can cause discomfort associated with insertion of the needle. Infusion of saline (5 mL.30min$^{-1}$) will be applied to minimise infection risk (flushing and locking eliminates potential nesting sites for microorganisms) and clotting. To avoid the saline affecting subsequent samples, 2.5 mL of blood will be drawn from the cannula before they are collected.

Other risks that have been accounted for are hypotension and loss of consciousness in the water. If at any point the participants BP is measured at ≥ 180/100 the trial will be terminated and the participant withdrawn from the study. If there is loss of consciousness in the water the participant will immediately be harnessed securely out. This can be done quickly as they will already be lying on the sling. Once they are out of the water and placed on a bed, their breathing will be checked and first aid administered. They will also be cooled via cold water spray and a fan. Additionally, their head will never go underwater as their head will be elevated above waterline throughout all submersions.

### 14.1 Participant Confidentiality

The study staff will ensure that the participants' anonymity is maintained. The participants will be identified only by initials and a participants ID number on the CRF and any electronic database. All documents will be stored securely and only accessible by study staff and authorised personnel. The study will comply with the Data Protection Act which requires data to be anonymised as soon as it is practical to do so.

### 14.2 Other Ethical Considerations

N/A

### 14.3 Declaration of Helsinki

The study protocol will be carried out in accordance with the Declaration of Helsinki.

### 14.4 ICH Guidelines for Good Clinical Practice

All research staff exposed to participants will be good clinical practice (GCP) trained and will be monitored by the sponsor to ensure adherence to GCP.

## 15. PATIENT PUBLIC INVOLVEMENT (PPI)

### 15.1 Study design

Evidence of patient, carer and public involvement (PCPI):

The chief investigator has a strong track record of PCPI work having been involved in PCPI award winning projects recently from the UK Stroke Forum (October 2016) for another acceptability and feasibility trial. The lay summary and patient facing documentation has been reviewed by our PCPI co-investigator.

### 15.2 Study implementation

Issues will be managed by the senior members of the research team.

### 15.3 Dissemination

We endeavour to disseminate findings through local groups, local/national media, research papers and international conferences.

## 16. FINANCING AND INSURANCE

### 16.1 Research Costs

The majority of consumables and personnel required for testing have been funded by the University of Portsmouth. All hardware and software is already available.

### 16.2 Service Support Costs

No service support costs are being sought.

### 16.3 Excess Treatment Costs

No excess treatment cost are being sought.

### 16.4 Study Sponsorship

This study is being sponsored by the University of Portsmouth.

## 17. TIMETABLE AND ORGANISATIONAL CHART

February 2018: Funding for consumables has been secured
July 2018: Application for ethical approval
October 2018 - June 2019: Participant recruitment
October 2018 - April 2020: Data collection
April 2020 – June 2020: Data analysis
June 2020: Study completion date
Post-June 2020: Dissemination of results through conference proceedings, journal submissions and practitioner outlets.

## 18. RESOURCES, EQUIPMENT AND PHYSICAL FACILITIES

In addition to the departmental support, and in order to facilitate the completion of the work, the research team will have full access to all of the necessary world-leading extreme environment facilities that are housed within the department estate given that they are pivotal to the success of the project. Specifically, this will enable temperature control within the facilities to ensure reproducibility. Finally, the department will ensure that full technical assistance is provided to enable the set-up of the laboratories and testing rooms.

## 19. DISSEMINATION AND OUTCOME

Results (estimates of effect sizes and confidence intervals) from this study will be utilised to define our primary outcome and to power a larger more complex study. The research is to be disseminated in internationally recognised, peer reviewed journals and at relevant scientific conferences, as well as contributing to Mr. Thomas James' PhD thesis. Furthermore, all screening data will be utilised as part of the patient's annual report, and distributed to clinicians and participants upon request.

## 20. REFERENCES


1. Ary, D. V., et al. (1986). "Patient perspective on factors contributing to nonadherence to diabetes regimen." Diabetes care **9**(2): 168-172.
2. Atalay, M., et al. (2004). "Exercise training modulates heat shock protein response in diabetic rats." Journal of Applied Physiology **97**(2): 605-611.
3. Bacchi, E., Negri, C., Targher, G., Faccioli, N., Lanza, M., Zoppini, G., . . . Moghetti, P. (2013). Both resistance training and aerobic training reduce hepatic fat content in type 2 diabetic subjects with nonalcoholic fatty liver disease (the RAED2 Randomized Trial). *Hepatology, 58*(4), 1287-1295.
4. Barnard, N., et al. (2000). "Acceptability of a therapeutic low-fat, vegan diet in premenopausal women." Journal of Nutrition Education **32**(6): 314-319.
5. Baron, A. D., et al. (1994). "Skeletal muscle blood flow independently modulates insulin-mediated glucose uptake." American Journal of Physiology-Endocrinology And Metabolism **266**(2): E248-E253.
6. Bathaie, S. Z., et al. (2010). "The effect of hot-tub therapy on serum Hsp70 level and its benefit on diabetic rats: a preliminary report." International journal of hyperthermia **26**(6): 577-585.
7. Benelam, B., & Wyness, L. (2010). Hydration and health: a review. *Nutrition Bulletin, 35*(1), 3-25.

8. Birnie, J. and J. Grayson (1952). "Observations on temperature distribution and liver blood flow in the rat." The Journal of physiology **116**(2): 189-201.
9. Blond, E., et al. (2011). "A new indirect calorimeter is accurate and reliable for measuring basal energy expenditure, thermic effect of food and substrate oxidation in obese and healthy subjects." European e-Journal of Clinical Nutrition and Metabolism **6**(1): e7-e15.
10. Braun, B., et al. (1995). "Effects of exercise intensity on insulin sensitivity in women with non-insulin-dependent diabetes mellitus." Journal of Applied Physiology **78**(1): 300-306.
11. Bruce, C. R., et al. (2003). "Intramuscular heat shock protein 72 and heme oxygenase-1 mRNA are reduced in patients with type 2 diabetes: evidence that insulin resistance is associated with a disturbed antioxidant defense mechanism." Diabetes **52**(9): 2338-2345.
12. Bruce, J. L., Price, B. D., Coleman, C. N., & Calderwood, S. K. (1993). Oxidative injury rapidly activates the heat shock transcription factor but fails to increase levels of heat shock proteins. *Cancer research, 53*(1), 12-15.
13. Brunt, V. E., et al. (2016). "Passive heat therapy improves endothelial function, arterial stiffness and blood pressure in sedentary humans." J Physiol **594**(18): 5329-5342.
14. Caldwell, J. N., et al. (2011). "The interaction of body armor, low-intensity exercise, and hot-humid conditions on physiological strain and cognitive function." Military medicine **176**(5): 488-493.
15. Cheng, A. Y. and I. G. Fantus (2005). "Oral antihyperglycemic therapy for type 2 diabetes mellitus." Canadian Medical Association Journal **172**(2): 213-226.
16. Cherrington, A. D. (1999). "Control of glucose uptake and release by the liver in vivo." Diabetes **48**(5): 1198.
17. Chung, J., et al. (2008). "HSP72 protects against obesity-induced insulin resistance." Proceedings of the National Academy of Sciences **105**(5): 1739-1744.
18. Clark, D. O. (1997). "Physical activity efficacy and effectiveness among older adults and minorities." Diabetes care **20**(7): 1176-1182.
19. Cockcroft, E. J., Williams, C. A., Jackman, S. R., Bassi, S., Armstrong, N., & Barker, A. R. (2018). A single bout of high-intensity interval exercise and work-matched moderate-intensity exercise has minimal effect on glucose tolerance and insulin sensitivity in 7-to 10-year-old boys. *Journal of sports sciences, 36*(2), 149-155.
20. Cohen, R. M., et al. (2008). "Red cell life span heterogeneity in hematologically normal people is sufficient to alter HbA1c." Blood **112**(10): 4284-4291.
21. Collaboration, E. R. F. (2010). "Diabetes mellitus, fasting blood glucose concentration, and risk of vascular disease: a collaborative meta-analysis of 102 prospective studies." The Lancet **375**(9733): 2215-2222.
22. Crandall, C., et al. (1999). "Effect of increasing central venous pressure during passive heating on skin blood flow." Journal of Applied Physiology **86**(2): 605-610.
23. Crandall, C., et al. (2008). "Effects of passive heating on central blood volume and ventricular dimensions in humans." The Journal of physiology **586**(1): 293-301.
24. Daousi, C., Casson, I., Gill, G., MacFarlane, I., Wilding, J., & Pinkney, J. (2006). Prevalence of obesity in type 2 diabetes in secondary care: association with cardiovascular risk factors. *Postgraduate medical journal, 82*(966), 280-284.
25. Diabetes prevention programme research group (DPPRG). (2002). Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. *New England Journal of Medicine, 346*(6), 393-403.

26. Dubé, J. J., Fleishman, K., Rousson, V., Goodpaster, B. H., & Amati, F. (2012). Exercise dose and insulin sensitivity: relevance for diabetes prevention. *Medicine and science in sports and exercise, 44*(5), 793.
27. Faulkner, S. H., et al. (2017). "The effect of passive heating on heat shock protein 70 and interleukin-6: A possible treatment tool for metabolic diseases?" Temperature (Austin) **4**(3): 292-304.
28. Febbraio, M. A. and B. K. Pedersen (2002). "Muscle-derived interleukin-6: mechanisms for activation and possible biological roles." The FASEB Journal **16**(11): 1335-1347.
29. Frayn, K. (1983). Calculation of substrate oxidation rates in vivo from gaseous exchange. *Journal of Applied Physiology, 55*(2), 628-634.
30. George, E. S., et al. (2013). "Chronic disease and sitting time in middle-aged Australian males: findings from the 45 and Up Study." International Journal of Behavioral Nutrition and Physical Activity **10**(1): 20.
31. Gibson, O. R., et al. (2014). "Extracellular Hsp72 concentration relates to a minimum endogenous criteria during acute exercise-heat exposure." Cell Stress and Chaperones **19**(3): 389-400.
32. Gillen, J., Little, J., Punthakee, Z., Tarnopolsky, M., Riddell, M., & Gibala, M. (2012). Acute high-intensity interval exercise reduces the postprandial glucose response and prevalence of hyperglycaemia in patients with type 2 diabetes. *Diabetes, Obesity and Metabolism, 14*(6), 575-577.
33. Glasgow, R. E., et al. (1997). "Personal-model beliefs and social-environmental barriers related to diabetes self-management." Diabetes care **20**(4): 556-561.
34. Group, U. P. D. S. (1998). "Effect of intensive blood-glucose control with metformin on complications in overweight patients with type 2 diabetes (UKPDS 34)." The Lancet **352**(9131): 854-865.
35. Gupte, A. A., et al. (2009). "Heat treatment improves glucose tolerance and prevents skeletal muscle insulin resistance in rats fed a high-fat diet." Diabetes **58**(3): 567-578.
36. Gupte, A. A., et al. (2010). "Acute heat treatment improves insulin-stimulated glucose uptake in aged skeletal muscle." Journal of Applied Physiology **110**(2): 451-457.
37. Hales, J., et al. (1979). "Regional distribution of blood flow in awake heat-stressed baboons." American Journal of Physiology-Heart and Circulatory Physiology **237**(6): H705-H712.
38. Hallsworth, K., Fattakhova, G., Hollingsworth, K. G., Thoma, C., Moore, S., Taylor, R., . . . Trenell, M. I. (2011). Resistance exercise reduces liver fat and its mediators in non-alcoholic fatty liver disease independent of weight loss. *Gut*, gut. 2011.242073.
39. Hayden, M. R., et al. (2005). "Type 2 diabetes mellitus as a conformational disease." Jop **6**(4): 287-302.
40. Heinonen, I., et al. (2011). "Local heating, but not indirect whole body heating, increases human skeletal muscle blood flow." Journal of Applied Physiology **111**(3): 818-824.
41. Hensold, J. O., Hunt, C. R., Calderwood, S. K., Housman, D. E., & Kingston, R. E. (1990). DNA binding of heat shock factor to the heat shock element is insufficient for transcriptional activation in murine erythroleukemia cells. *Molecular and cellular biology, 10*(4), 1600-1608.
42. Henstridge, D. C., et al. (2010). "The relationship between heat shock protein 72 expression in skeletal muscle and insulin sensitivity is dependent on adiposity." Metabolism-Clinical and Experimental **59**(11): 1556-1561.
43. Henstridge, D. C., et al. (2014). "Activating HSP72 in rodent skeletal muscle increases mitochondrial number and oxidative capacity and decreases insulin resistance." Diabetes **63**(6): 1881-1894.

44. Hermanns, N., et al. (2014). "Effect of local heating on postprandial blood glucose excursions using the InsuPad device: results of an outpatient crossover study." Journal of diabetes science and technology **8**(6): 1126-1132.
45. Hex, N., et al. (2012). "Estimating the current and future costs of Type 1 and Type 2 diabetes in the UK, including direct health costs and indirect societal and productivity costs." Diabetic Medicine **29**(7): 855-862.
46. Hooper, P. L. (1999). "Hot-tub therapy for type 2 diabetes mellitus." New England Journal of Medicine **341**(12): 924-925.
47. Hooper, P. L. and P. L. Hooper (2009). "Inflammation, heat shock proteins, and type 2 diabetes." Cell Stress Chaperones **14**(2): 113-115.
48. Hotamisligil, G. S. (2006). "Inflammation and metabolic disorders." Nature **444**(7121): 860.
49. Hundal, R. S., et al. (2002). "Mechanism by which high-dose aspirin improves glucose metabolism in type 2 diabetes." The Journal of clinical investigation **109**(10): 1321-1326.
50. Hunter-Lavin, C., et al. (2004). "Hsp70 release from peripheral blood mononuclear cells." Biochemical and biophysical research communications **324**(2): 511-517.
51. Hwang, S., et al. (2009). "Sequential preoperative ipsilateral hepatic vein embolization after portal vein embolization to induce further liver regeneration in patients with hepatobiliary malignancy." Annals of surgery **249**(4): 608-616.
52. Iiyama et al. (2007). "Effects of whole body warm water immersion on indocyanine green (ICG) excretion test in healthy human." 日本温泉気候物理医学会雑誌 **70**(4): 215-222.
53. Jelleyman, C., Yates, T., O'Donovan, G., Gray, L. J., King, J. A., Khunti, K., & Davies, M. J. (2015). The effects of high-intensity interval training on glucose regulation and insulin resistance: a meta-analysis. *Obesity reviews, 16*(11), 942-961.
54. Jorge, M. L., et al. (2011). "The effects of aerobic, resistance, and combined exercise on metabolic control, inflammatory markers, adipocytokines, and muscle insulin signaling in patients with type 2 diabetes mellitus." Metabolism **60**(9): 1244-1252.
55. Kadoglou, N. P., et al. (2007). "The anti-inflammatory effects of exercise training in patients with type 2 diabetes mellitus." European Journal of Cardiovascular Prevention & Rehabilitation **14**(6): 837-843.
56. Kang, J., et al. (1996). "Effect of Exercise Intensity on Glucose and Insulin Metabolism in Obese Individuals and Obese NIDDM Patients." Diabetes care **19**(4): 341-349.
57. Katz, A., et al. (2000). "Quantitative insulin sensitivity check index: a simple, accurate method for assessing insulin sensitivity in humans." The Journal of Clinical Endocrinology & Metabolism **85**(7): 2402-2410.
58. Kavanagh, K., et al. (2009). "Tissue-specific regulation and expression of heat shock proteins in type 2 diabetic monkeys." Cell Stress and Chaperones **14**(3): 291-299.
59. Kelly, T., et al. (2008). "Global burden of obesity in 2005 and projections to 2030." International journal of obesity **32**(9): 1431.
60. Kitsios, K., et al. (2011). "Glycemia and cardiovascular risk: challenging evidence based medicine." Hippokratia **15**(3): 199.
61. Koda, M., et al. (1995). "Effects of bathing in hot water on portal hemodynamics in healthy subjects and in patients with compensated liver cirrhosis." Internal medicine **34**(7): 628-631.
62. Kokura, S., et al. (2007). "Whole body hyperthermia improves obesity-induced insulin resistance in diabetic mice." International journal of hyperthermia **23**(3): 259-265.

63. Kondo, S., et al. (2012). "Evaluation of a wound dressing composed of hyaluronic acid and collagen sponge containing epidermal growth factor in diabetic mice." Journal of Biomaterials Science, Polymer Edition **23**(13): 1729-1740.
64. Krause, M., et al. (2015). "The chaperone balance hypothesis: the importance of the extracellular to intracellular HSP70 ratio to inflammation-driven type 2 diabetes, the effect of exercise, and the implications for clinical management." Mediators of inflammation **2015**.
65. Kurucz, I., et al. (2002). "Decreased expression of heat shock protein 72 in skeletal muscle of patients with type 2 diabetes correlates with insulin resistance." Diabetes **51**(4): 1102-1109.
66. Laborde, S., et al. (2017). "Heart rate variability and cardiac vagal tone in psychophysiological research–recommendations for experiment planning, data analysis, and data reporting." Frontiers in psychology **8**: 213.
67. Lautt, W. W., et al. (1990). "Quantitation of the hepatic arterial buffer response to graded changes in portal blood flow." Gastroenterology **98**(4): 1024-1028.
68. Li, M., et al. (2008). "Treatment of obese diabetic mice with a heme oxygenase inducer reduces visceral and subcutaneous adiposity, increases adiponectin levels, and improves insulin sensitivity and glucose tolerance." Diabetes **57**(6): 1526-1535.
69. Little, J. P., Gillen, J. B., Percival, M. E., Safdar, A., Tarnopolsky, M. A., Punthakee, Z., . . . Gibala, M. J. (2011). Low-volume high-intensity interval training reduces hyperglycemia and increases muscle mitochondrial capacity in patients with type 2 diabetes. *Journal of Applied Physiology, 111*(6), 1554-1560.
70. Magkos, F., Tsekouras, Y., Kavouras, S. A., Mittendorfer, B., & Sidossis, L. S. (2008). Improved insulin sensitivity after a single bout of exercise is curvilinearly related to exercise energy expenditure. *Clinical Science, 114*(1), 59-64.
71. Manders, R., Van Dijk, J., & Van Loon, L. (2010). Low-intensity exercise reduces the prevalence of hyperglycemia in type 2 diabetes. *Medicine and science in sports and exercise, 42*(2), 219-225.
72. Morino, S., et al. (2008). "Mild electrical stimulation with heat shock ameliorates insulin resistance via enhanced insulin signaling." PLoS One **3**(12): e4068.
73. Nathan, D., et al. (2006). "Management of hyperglycaemia in type 2 diabetes: a consensus algorithm for the initiation and adjustment of therapy." Diabetologia **49**(8): 1711-1721.
74. Newsom, S. A., Everett, A. C., Hinko, A., & Horowitz, J. F. (2013). A single session of low-intensity exercise is sufficient to enhance insulin sensitivity into the next day in obese adults. *Diabetes care, 36*(9), 2516-2522.
75. O'gorman, D., et al. (2006). "Exercise training increases insulin-stimulated glucose disposal and GLUT4 (SLC2A4) protein content in patients with type 2 diabetes." Diabetologia **49**(12): 2983-2992.
76. Ogurtsova, K., et al. (2017). "IDF Diabetes Atlas: Global estimates for the prevalence of diabetes for 2015 and 2040." Diabetes research and clinical practice **128**: 40-50.
77. Olsen, R. H., Krogh-Madsen, R., Thomsen, C., Booth, F. W., & Pedersen, B. K. (2008). Metabolic responses to reduced daily steps in healthy nonexercising men. *Jama, 299*(11), 1261-1263.
78. Organization, W. H. (2016). Global report on diabetes, World Health Organization.
79. Otten, J., et al. (2014). "Surrogate measures of insulin sensitivity vs the hyperinsulinaemic-euglycaemic clamp: a meta-analysis." Diabetologia **57**(9): 1781-1788.
80. Papanas, N., et al. (2011). "Peripheral neuropathy is associated with increased serum levels of uric acid in type 2 diabetes mellitus." Angiology **62**(4): 291-295.

81. Periard, J. D., et al. (2011). "Central and peripheral fatigue during passive and exercise-induced hyperthermia." Med Sci Sports Exerc **43**(9): 1657-1665.
82. Raz, I., et al. (2009). "Effect of a local heating device on insulin and glucose pharmacokinetic profiles in an open-label, randomized, two-period, one-way crossover study in patients with type 1 diabetes using continuous subcutaneous insulin infusion." Clinical therapeutics **31**(5): 980-987.
83. Rivas, E., et al. (2016). "An acute bout of whole body passive hyperthermia increases plasma leptin, but does not alter glucose or insulin responses in obese type 2 diabetics and healthy adults." J Therm Biol **59**: 26-33.
84. Roberts, C. K., Hevener, A. L., & Barnard, R. J. (2013). Metabolic syndrome and insulin resistance: underlying causes and modification by exercise training. *Comprehensive Physiology*.
85. Rodrigues-Krause, J., et al. (2012). "Divergence of intracellular and extracellular HSP72 in type 2 diabetes: does fat matter?" Cell Stress and Chaperones **17**(3): 293-302.
86. Rodrigues-Krause, J., et al. (2012). "Divergence of intracellular and extracellular HSP72 in type 2 diabetes: does fat matter?" Cell Stress and Chaperones **17**(3): 293-302.
87. Rowell, L. B., et al. (1970). "Redistribution of blood flow during sustained high skin temperature in resting man." Journal of Applied Physiology **28**(4): 415-420.
88. Shaw, J. E., Sicree, R. A., & Zimmet, P. Z. (2010). Global estimates of the prevalence of diabetes for 2010 and 2030. *Diabetes research and clinical practice*, *87*(1), 4-14.
89. Shoelson, S. E., et al. (2006). "Inflammation and insulin resistance." The Journal of clinical investigation **116**(7): 1793-1801.
90. Shoelson, S., et al. (2003). "Inflammation and the IKKβ/IκB/NF-κB axis in obesity-and diet-induced insulin resistance." International journal of obesity **27**(S3): S49.
91. Siddle, K. (2011). "Signalling by insulin and IGF receptors: supporting acts and new players." Journal of molecular endocrinology **47**(1): R1-R10.
92. Stamler, J., et al. (1993). "Diabetes, other risk factors, and 12-yr cardiovascular mortality for men screened in the Multiple Risk Factor Intervention Trial." Diabetes care **16**(2): 434-444.
93. Steensberg, A., et al. (2003). "IL-6 enhances plasma IL-1ra, IL-10, and cortisol in humans." American Journal of Physiology-Endocrinology And Metabolism **285**(2): E433-E437.
94. Stice, J. P. and A. A. Knowlton (2008). "Estrogen, NFκB, and the heat shock response." Molecular Medicine **14**(7-8): 517.
95. Stratton, I. M., et al. (2000). "Association of glycaemia with macrovascular and microvascular complications of type 2 diabetes (UKPDS 35): prospective observational study." Bmj **321**(7258): 405-412.
96. Stumvoll, M., et al. (2000). "Use of the oral glucose tolerance test to assess insulin release and insulin sensitivity." Diabetes care **23**(3): 295-301.
97. Stunkard, A. J. and S. Messick (1985). "The three-factor eating questionnaire to measure dietary restraint, disinhibition and hunger." Journal of psychosomatic research **29**(1): 71-83.
98. Tsuzuki, T., et al. (2017). "Attenuation of exercise-induced heat shock protein 72 expression blunts improvements in whole-body insulin resistance in rats with type 2 diabetes." Cell Stress and Chaperones **22**(2): 263-269.
99. Voulgari, C., et al. (2013). "Exercise improves cardiac autonomic function in obesity and diabetes." Metabolism **62**(5): 609-621.

100. Wang, X., Patterson, B. W., Smith, G. I., Kampelman, J., Reeds, D. N., Sullivan, S. A., & Mittendorfer, B. (2013). A~ 60-min brisk walk increases insulin-stimulated glucose disposal but has no effect on hepatic and adipose tissue insulin sensitivity in older women. *Journal of Applied Physiology, 114*(11), 1563-1568.
101. Weyer, C., et al. (2001). "Hypoadiponectinemia in obesity and type 2 diabetes: close association with insulin resistance and hyperinsulinemia." The Journal of Clinical Endocrinology & Metabolism **86**(5): 1930-1935.
102. Wojtaszewski, J. F., Nielsen, J. N., & Richter, E. A. (2002). Invited review: effect of acute exercise on insulin signaling and action in humans. *Journal of Applied Physiology, 93*(1), 384-392.
103. Yuan, M., et al. (2001). "Reversal of obesity-and diet-induced insulin resistance with salicylates or targeted disruption of Ikkβ." Science **293**(5535): 1673-1677.
104. Zhang, H., et al. (2004). "Thermal sensation and comfort in transient non-uniform thermal environments." European journal of applied physiology **92**(6): 728-733.
105. Zoungas, S., et al. (2017). "Effects of intensive glucose control on microvascular outcomes in patients with type 2 diabetes: a meta-analysis of individual participant data from randomised controlled trials." The Lancet Diabetes & Endocrinology **5**(6): 431-437.

## 21. APPENDIX 1 SCHEDULE OF PROCEDURES

## 22. APPENDIX 2 STUDY FLOW CHART

## 23. APPENDIX 3 PARTICIPANT INFORMATION SHEET

PUT PHT TEMPLATE HERE

## 24. APPENDIX 4 INFORMED CONSENT FORM

PUT PHT TEMPLATE HERE