The effect of taurine supplementation on physiological and thermoregulatory responses in humans during rest and exercise in hot environmental conditions

Jennifer Sarah Peel

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Abstract

This thesis details the meta-analytical and experimental work conducted to investigate the effects of dietary supplements, specifically taurine, on physiological and thermoregulatory responses in humans during heat exposure. Meta-analysis determined that the ergogenic effects of many dietary supplements on endurance exercise performance appear affected by the heat. Supplements established to be efficacious in thermoneutral conditions, such as caffeine and creatine provided no performance benefit, while amino acids (e.g. taurine) demonstrated the greatest ergogenicity. Of the supplements meta-analysed for their thermoregulatory effects, several amino acids, anti-oxidants and anti-inflammatories, and those affecting fluid balance, offered the greatest benefits during heat exposure. Conversely, supplements enhancing nitric oxide bioavailability had no effect on thermal balance, and caffeine induced a thermogenic effect when ingested in the heat. Overall, taurine had the greatest performance and thermoregulatory responses, and was, therefore, selected as the focus of the subsequent empirical data chapters. Within the experimental studies of the thesis, taurine supplementation augmented thermal sweating during fixed metabolic heat production in hot conditions, including increased whole-body sweat loss, local sweat rate and sweat gland activation, alongside enhancing cutaneous vasodilation. Greater thermal sweating translated to heightened evaporative heat dissipation and reduced heat storage, as modelled by partitional calorimetry. Improved thermal tolerance was also observed, through a delayed transition to uncompensable heat stress. Drivers of the thermal sweating response and the measurement techniques used to assess these were established to be sufficiently reliable to control thermal sweating and detect likely changes, respectively. This indicates that the findings regarding taurine's effects on thermal sweating are genuine and unaffected by these influencing factors. Taurine may exert these thermoregulatory effects through its vaso-active and osmoregulatory roles, though this requires further investigation. Nevertheless, taurine may offer a potential dietary supplementation strategy to support thermoregulation in hot environmental conditions that permit dry and evaporative heat transfer.

Declarations and Statements

This work has not previously been accepted in substance for any degree and is not being concurrently submitted in candidature for any degree.

Signed……  …… (candidate)

Date……28/05/2024……

STATEMENT 1

This thesis is the result of my own investigations, except where otherwise stated. Other sources are acknowledged by footnotes giving explicit references. A bibliography is appended.

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STATEMENT 2

I hereby give consent for my thesis, if accepted, to be available for photocopying, electronic sharing and inter-library loan, and for the title and summary to be made available to outside organisations by Swansea University.

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STATEMENT 3

The University's ethical procedures have been followed and, where appropriate, that ethical approval has been granted.

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Thesis Contributions

Jennifer Peel and Dr Mark Waldron designed the studies, with support from Prof. Melitta McNarry, Dr Shane Heffernan, Rene Nevola and Prof. Liam Kilduff. Jennifer Peel conducted the data collection and analysed the data, with support from Dr Mark Waldron. Dr Ed Dudley, Kathryn Coates and Alanna Thomas conducted the HPLC analysis (Chapters Seven and Eight). Jennifer Peel wrote the thesis, Dr Mark Waldron reviewed the thesis and Prof. Melitta McNarry, Dr Shane Heffernan, Rene Nevola and Prof. Liam Kilduff reviewed all Chapters of the thesis.

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List of figures and tables

Abbreviations

1,3,7-trimethylxanthine	Caffeine
ACSM	American College of Sports Medicine
A_D	Body surface area
ADH	Antidiuretic hormone
ANOVA	Analysis of variance
A_r/A_D	Fraction of the body surface participating in radiative heat transfer
AVP	Arginine vasopressin
BCAAs	Branched-chain amino acids
BSA	Body surface area
Ca^{2+}	Calcium
CBS	Cystathionine-β-synthase
CI	Confidence Intervals
Cl^-	Chloride
C_{res}	Convective respiratory heat loss
CSE	Cystathionine γ-lyase
C_{skin}	Convection
CV%	Coefficient of variation
CVC	Cutaneous vascular conductance
D	Artery diameter
$D_{baseline}$	Resting artery diameter
DBP	Diastolic blood pressure
D_{max}	Maximum artery diameter
ECG	Electrocardiogram
ECT	End core temperature
EDTA	Ethylenediaminetetraacetic acid
$\dot{E}_{max}$	Maximal evaporative heat transfer capacity
$\dot{E}_{req}$	Evaporation required to maintain heat balance
E_{res}	Evaporative respiratory heat loss
$\dot{E}_{skin}$	Evaporative heat transfer
f_{cl}	Clothing area factor
FMD	Flow-mediated dilation

GABA	Gamma-aminobutyric acid
GI	Gastrointestinal
h	Combined convective heat transfer coefficient
H_2S	Hydrogen sulphide
h_c	Convective heat transfer coefficient
$\dot{H}_{dry,skin}$	Dry heat exchange at the skin
h_e	Evaporative heat transfer coefficient
HHB	Deoxyhaemoglobin concentration
HPLC	High-performance liquid chromatography
$\dot{H}_{prod}$	Metabolic heat production
h_r	Radiative heat transfer coefficient
HR	Heart rate
$\dot{H}_{res}$	Respiratory heat loss
HR_{max}	Maximum heart rate
IHG	Isometric handgrip
IOC	International Olympic Committee
IST	Intermittent sprint tests
K^+	Potassium
K_{skin}	Conduction
LSR	Local sweat rate
$\dot{M}$	Metabolic energy expenditure
MAP	Mean arterial pressure
MeSH	Medical subject headings
MVC	Maximum voluntary contraction
n	Sample size
Na^+	Sodium
NIRS	Near-infrared spectroscopy
NO	Nitric oxide
NO_3^-	Dietary nitrate
NS	Non-significant
O_2	Oxygen
P_a	Vapor pressure of inspired air
PAC1	Pituitary adenylate cyclase-activating peptide 1 receptor
P_{crit}	Breakpoint of uncompensability

PPO	Peak power output
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analysis
$P_{skin,sat}$	Vapour pressure at the skin surface while saturated in sweat
$\dot{Q}$	Cardiac output
R_{cl}	Dry heat transfer of clothing
$R_{e,cl}$	Evaporative resistance of clothing
RER	Respiratory exchange ratio
RH	Relative humidity
RM-ANOVA	Repeated measures analysis of variance
ROS	Reactive oxygen species
RPE	Rating of perceiving exertion
R_{skin}	Radiation
SBP	Systolic blood pressure
SCT	Submaximal core temperature
SD	Standard deviation
SE/SEM	Standard error
S_{eff}	Sweating efficiency
SGA	Sweat gland activation
SH	Specific heat of the blood
SkBF	Skin blood flow
SMD	Standardised mean difference
SPO_2	Oxygen saturation
SV	Stroke volume
t_a	Ambient air temperature
T_{arm}	Arm skin temperature
TauT	Taurine transporters
TC	Thermal comfort
T_{calf}	Calf skin temperature
T_{chest}	Chest skin temperature
T_{core}	Core temperature
T_{db}	Dry-bulb temperature
tHB	Total haemoglobin concentration
t_o	Operative temperature

t_r	Radiant temperature
TS	Thermal sensation
TSI%	Tissue saturation index
T_{sk}	Skin temperature
TT	Time-trial
TTE	Time-to-exhaustion
T_{thigh}	Thigh skin temperature
UEFA	Union of European Football Associations
V2 receptors	Vasopressin subtype 2 receptors
V_{air}	Ambient air velocity
$\dot{V}CO_2$	Carbon dioxide production
VE	Minute ventilation
V_{mean}	Mean blood velocity
$\dot{V}O_2$	Oxygen uptake
$\dot{V}O_{2max}$	Maximal oxygen uptake
$\dot{V}O_{2peak}$	Peak oxygen uptake
VPAC	Vaso-active intestinal peptide receptors
WBGT	Wet-bulb globe temperature
WBSL	Whole-body sweat loss
WBSR	Whole-body sweat rate
Wk	Mechanical/external work
WR	Work rate
ε	Non-dimensional emissivity of the body surface
λ	Latent heat vaporisation of sweat
σ	Stefan-Boltzmann constant
ω_{req}	Required skin wettedness

1. Chapter One – Introduction

Global temperatures are rising, and the occurrence, severity and duration of heat waves are increasing (Lee et al., 2023), leading to a greater proportion of the population becoming exposed to periods of extreme heat (Marx et al., 2021). Further, many athletic events and international competitions are conducted in hot and/or humid conditions (Ely et al., 2008; Racinais et al., 2015) and military training and operations are also often performed in extreme environments (Parsons et al., 2019; World & Booth, 2008). Such conditions can induce heat strain, due to reduced avenues of heat dissipation (Cramer & Jay, 2016; Gagge & Gonzalez, 1996) and increased metabolic heat production ($\dot{H}_{prod}$), if performing physical activity (Coris et al., 2004; Lundgren et al., 2013; Nadel et al., 1977). If heat is not sufficiently dissipated from the body to the environment, heat storage ensues (uncompensable heat stress), causing rises in core temperature (T_{core}) and, if prolonged, heat strain (Cramer & Jay, 2016). This can lead to impaired exercise performance (Galloway & Maughan, 1997; Hargreaves, 2008; Junge et al., 2016; Maughan et al., 2012) and adverse health outcomes (i.e. heat illness, cardiovascular events and even death; Gasparrini & Armstrong, 2011; Jingesi et al., 2023; Liu et al., 2022; Pradhan et al., 2019; Weisskopf et al., 2002; Zhang et al., 2020).

In hot environmental conditions and during exercise, evaporation of fluid off the skin's surface represents the primary heat loss pathway (Nielsen, 1938; Wenger, 1972). This occurs secondary to sweating and is largely dependent on sweating efficiency and the ambient vapour pressure gradient present between the skin and air (Che Muhamed et al., 2016; Gagge & Gonzalez, 1996; Parsons, 2007). A larger temperature and vapour pressure gradient allows for greater dry (conduction, convection and radiation) and evaporative heat dissipation, respectively (Gagge & Gonzalez, 1996). Therefore, in hot and humid conditions, where this is reduced, compensatory heat loss mechanisms are required to augment heat dissipation (i.e. sweating and cutaneous vasodilation). As such, eccrine sweat production is an essential physiological mechanism for the maintenance of thermal balance in these environments (Marino et al., 2000; Sawka & Young, 2006). In healthy individuals, thermal sweating is stimulated in response to elevated core and/or skin temperature (T_{sk}; Hammel & Pierce, 1968; Werner, 1981), but conceptually, sweat production appears to be driven by $\dot{H}_{prod}$ and the rate of evaporation required to maintain heat balance ($\dot{E}_{req}$; Cramer & Jay, 2014, 2016; Gagnon et al., 2013; Chapter Six). Thus, manipulation of factors affecting calorimetric components, such as $\dot{H}_{prod}$ or any heat loss avenue, will affect thermal balance.

There are three factors which can be modified to improve thermal balance in hot conditions: lowered $\dot{H}_{prod}$, enhanced cutaneous vasodilation (i.e. dry heat loss) and sweating (i.e. latent heat transfer; Benzinger et al., 1961a; Gagge & Gonzales, 1996; Wendt et al., 2007). Indeed,

various interventions, such as endurance training and heat acclimation can modify thermoregulatory responses (Périard et al., 2021), improving an individual's ability to activate thermoregulatory defences and tolerate hot environmental conditions (Périard et al., 2016; Ravanelli et al., 2018; Wenger, 1972). Sweating is the primary manipulable pathway, with a hastened sweating onset and increased sweat rate and sweat gland activation (SGA) often observed. Other adaptations include improved skin blood flow (SkBF), plasma volume expansion and lower resting T_{core} and oxygen cost of exercise (Klous et al., 2020; Lorenzo et al., 2010; Périard et al., 2016; Poirier et al., 2016; Ravanelli et al., 2018; Rivas et al., 2017). More recently, other more acute interventions, such as dietary supplementation, have been proposed to influence thermoregulatory responses and thermal balance when ingested in hot conditions (Jardine et al., 2023; Twycross-Lewis et al., 2016; Chapter Four). Indeed, oral taurine supplementation has been demonstrated to prolong exercise time to exhaustion by approximately 10 to 15% in the heat, while concomitantly reducing end T_{core} (38.1°C *vs* 38.5°C [Page et al., 2019] & 38.2°C *vs* 38.4°C [Yu et al., 2024]). An earlier onset of sweating and an increased sweat rate (~13%) have also been observed in these conditions (Page et al., 2019).

Taurine is a sulphur containing amino acid, involved in numerous physiological processes, such as neuromodulation, osmoregulation, anti-oxidation, anti-inflammation and regulation of mitochondrial metabolism and calcium homeostasis (Huxtable 1992; Schaffer et al., 2010). It has been shown to be ergogenic for endurance exercise performance in thermoneutral conditions (Balshaw et al., 2013; Waldron et al., 2019; Waldron et al., 2018a; Zhang et al., 2004), likely through effects on sarcoplasmic reticulum Ca^{+} handling (Dutka et al., 2014; Hamilton et al., 2006), anti-oxidation (Hansen et al., 2006; Hansen et al., 2010; Jong et al., 2021; Schaffer et al., 2022; Zhang et al., 2004) and/or alterations in substrate utilisation (Rutherford et al., 2010; Simmonds et al., 2022). The magnitude of these responses reflects those reported following heat acclimation (Waldron et al., 2021). Many of these ascribed biological roles have the potential to improve thermoregulatory capacity during heat exposure (i.e. via enhanced fluid availability; Huxtable, 1992). Taurine also has vaso-active properties, inducing vasodilation in *in-vitro* rodent arteries (Ulusoy et al., 2017; Yildiz & Ulusoy, 2022). Theoretically, taurine's effects on osmoregulation and vascular function, for example, may augment sweating and cutaneous vasodilation, thus increasing dry and evaporative heat transfer at the periphery and maintaining thermal balance. This provides potential explanations for the finding that supplementation elicits a thermoregulatory benefit during exercising heat stress (Page et al., 2019; Yu et al., 2024).

The effects of various dietary supplements on thermoregulatory responses and thermal balance during heat exposure have not been sufficiently established. Moreover, only two studies have been conducted investigating the thermoregulatory effects of taurine ingestion in

the heat (Page et al., 2019; Yu et al., 2024). Therein, there was limited control of heat loss and gain avenues and taurine was ingested as a single pre-exercise supplement, which could theoretically restrict some of its mechanistic roles. Therefore, taurine's effects on sweating and vasodilation during heat exposure and subsequent influence on avenues of heat transfer have not been thoroughly characterised. Further research is required for corroboration of findings, to offer additional insight and provide preliminary mechanistic understanding. The work presented within this thesis systematically investigated the effects of various dietary supplements on endurance exercise performance, T_{core} and sweating responses in the heat. More specifically, the effects of oral taurine supplementation on multiple measures of thermal sweating, vascular function, heat transfer, as modelled by partitional calorimetry, thermal tolerance and cardiometabolic responses were evaluated in an experimental setting. Greater knowledge regarding the role of dietary supplements in aiding thermoregulation in hot environmental conditions is necessary, as it is possible that this could be used as a future strategy to reduce the risk of heat strain and, perhaps, heat illness. This has potential implications for individuals exposed to environmental heat stress, such as the general population living in hot climates and occupational workers, military personnel and athletes performing in hot conditions.

1.1. Chapter Summary

The overarching aim of this thesis was to evaluate the effects of an orally administered dietary supplement (taurine) on physiological and thermoregulatory responses during heat exposure. As such, the current thesis contains an Introduction, detailing the context and purpose of the research within this thesis (Chapter One), a Literature Review, including information on heat transfer, human thermoregulation and potential influence of the dietary supplement taurine on thermoregulatory function, alongside highlighting key gaps within the understanding of how taurine supplementation may provide a benefit to thermoregulation during heat exposure (Chapter Two). This is followed by a list of all equations used within this research (Chapter Three). Also included are five data chapters, with the first two consisting of meta-analyses of various dietary supplements and their effects on exercise performance, T_{core} and sweating responses during heat exposure (Chapters Four and Five). The third data chapter is a reliability study of various measures of the sweating response (Chapter Six) and the final two data chapters focus on the effects of oral taurine supplementation on thermoregulatory responses during exercise and at rest in the heat (Chapter Seven and Eight). The final chapter of the thesis contains a General Summary and Discussion (Chapter Nine).

The research aims and hypotheses of each data chapter are outlined below:

Chapter Four: The effect of dietary supplements on endurance exercise performance and core temperature in hot environments: a meta-analysis and meta-regression.

Aims: To investigate the effects of selected dietary supplements on endurance performance in the heat, as well as the associated T_{core} responses.

Chapter Five: The effect of dietary supplements on core temperature and sweating responses in hot environmental conditions: a meta-analysis and meta-regression.

Aims: To investigate the effects of all known orally administered dietary supplements on T_{core} and sweating responses in the heat.

Chapter Six: Measurement of thermal sweating at rest and steady-state exercise in healthy adults: Inter-day reliability and relationships with components of partitional calorimetry.

Aims: To establish the inter-day reliability of: i) the modified iodine-paper technique for the measurement of SGA using two separate assessment methods (sweat gland counting and surface area covered); ii) the absorbent patch technique for the measurement of local sweat rate (LSR); and iii) pre- *vs* post-exercise body mass changes for measurement of whole-body sweat loss (WBSL). Finally, the relationship between all measurements of thermal sweating and both metabolic heat production and evaporative requirement for heat balance was assessed to establish the construct validity of these measurements.

Chapter Seven: The effect of eight-days oral taurine supplementation on thermoregulation during low-intensity exercise at fixed heat production in hot conditions of incremental humidity

Aims: To determine the effect of an eight-day taurine supplementation period on T_{core} and sweating responses (WBSL, LSR and SGA), calorimetric heat transfers components (evaporation at the skin, heat storage), delta plasma volume, and plasma taurine concentrations during prolonged low-intensity exercise of a fixed metabolic heat production in the heat at both fixed and increasing vapour pressure.

Hypotheses: Taurine supplementation would: i) induce greater sweating responses across the exercise protocol; ii) delay the increase in T_{core} during the period of increasing vapour pressure (transition to an uncompensable environment); iii) increase plasma volume and; iv) result in

greater evaporative heat transfer and reduced heat storage, as modelled by partitional calorimetry.

Chapter Eight: The effect of oral taurine supplementation on thermoregulatory and cardiometabolic responses to passive heat exposure

Aims: To determine the effect of an eight-day taurine supplementation period on T_{core}, vascular (blood pressure, SkBF, brachial artery diameter and blood flow), sweating (WBSL and LSR), cardiac (cardiac output [$\dot{Q}$], stroke volume [SV] and heart rate [HR]) and metabolic (oxygen consumption at the muscle) responses and plasma taurine concentrations during passive heating.

Hypotheses: Taurine supplementation would: i) induce greater sweating responses (WBSL and LSR); ii) induce peripheral vasodilation (brachial artery diameter and SkBF); iii) increase $\dot{Q}$ and SV, and lower blood pressure; iv) lower T_{core}.

2. Chapter Two – Literature review

2.1. Heat transfer

As mammals, humans are thermal regulators, where the aim is to maintain a stable T_{core} of approximately 37°C (range 35 to 40°C), through the exchange of heat between the body and the environment (Cannon, 1929; Lim et al., 2008; Moran & Mendal, 2002). Humans possess a large circulatory network, which transports and distributes metabolically derived and exogenous heat around the body, and our surface (i.e. skin) has heat exchange capabilities with our environment (Forster et al., 1946; He et al., 2003). Regulatory processes which modify internal heat production and external heat transfer can be activated if homeostatic mechanisms are challenged (Benzinger, 1969). There must be a balance between internal heat production and external heat dissipation in order to maintain thermal stability and ensure T_{core} is kept within an acceptable boundary (Lim et al., 2008). This occurs via the heat exchange avenues of radiation, conduction, convection and evaporation (Parsons, 2014) and is governed by the laws of thermodynamics, which define the principles of heat transfer (Taylor et al., 2014).

2.1.1. Avenues of heat transfer

How humans generate and exchange heat with the environment is best represented by the heat balance equation.

$$S = \dot{M} - Wk \pm K \pm \dot{R} \pm \dot{C} - \dot{E}\ [W] \qquad \text{(equation 1)}$$

Where: S is body heat storage; $\dot{M}$ is metabolic energy expenditure; Wk is mechanical/external work; K, $\dot{R}$ and $\dot{C}$ are conductive, radiative and convective heat transfer (dry heat transfer), respectively; and $\dot{E}$ is evaporative heat dissipation (latent heat transfer; Cramer & Jay, 2019).

2.1.1.1. Metabolic heat production

Metabolic heat production ($\dot{M}$ – Wk) is the difference between metabolic rate ($\dot{M}$) and the energy used to create external work (Wk) and always represents a source of heat gain (Kenny & Jay, 2011). Metabolic rate is the rate of energy released from carbohydrate (CHO), fat and amino acid catabolism during adenosine triphosphate (ATP) resynthesis, used for numerous cellular activities, such as muscle contractions (Cramer & Jay, 2016). Humans are relatively inefficient at the process of performing external work (i.e. muscle contractions) and, therefore,

approximately 80 to 100% of metabolic energy is released as heat into the body (Kenny & Jay, 2011). At rest and during low-moderate intensity exercise, the majority of metabolic energy is derived from oxidative pathways and is, therefore, proportional to the rate of oxygen consumption ($\dot{V}O_2$; Cramer & Jay, 2016). Consequently, metabolic rate ($\dot{M}$) can be estimated from $\dot{V}O_2$ and respiratory exchange ratio (RER), which accounts for calorific differences between carbohydrate and fat oxidation (Murgatroyd et al., 1993), and is termed 'indirect calorimetry'. Likewise, in laboratory settings, external work can be tightly regulated - using either an ergometer or treadmill - and measured (Atkins & Nicholson, 1963; Snellen, 1960). Therefore, through indirect calorimetry and ergometric values, metabolic heat production can be estimated as the difference between these ($\dot{M}$ – Wk). During exercise there is an increase in oxygen consumption ($\dot{V}O_2$) to meet the energetic demands of the active muscles, which drives $\dot{H}_{prod}$ upwards and, as such, $\dot{H}_{prod}$ rises with exercise intensity (Cramer & Jay, 2016). Heat production at rest, while seated, is approximately 85 W or 44 W/m^2 (Åstrand & Rodahl, 1970; Levine et al., 2000) and can range from there to 1500 W or 770 W/m^2 during heavy exercise (Cramer & Jay, 2016; Gaesser & Brooks, 1975).

2.1.1.2. Dry heat transfer

2.1.1.2.1. Radiation

Thermal radiation is the rate of heat transfer between bodies in the form of electromagnetic waves and is not affected by the intervening air or the medium it is transferred through (Kerslake, 1972). All matter and, therefore, objects emit and absorb thermal radiation, and this heat transfer is dependent on the involved bodies surface temperature and emissivity (ability to absorb or emit radiation; IUPS Thermal Commission, 2001). In humans, thermal radiation leads to heat loss if T_{sk} exceeds ambient radiant temperature and heat gain if T_{sk} is exceeded by ambient radiant temperature. Generally, ambient radiant temperature equals air temperature if not exposed to the sun, as solar radiation is a large source of radiant energy (Kenny & Jay, 2011). Therefore, thermal radiation is considered to be relatively minor if indoors (e.g. -25 W/m^2 at 25°C and 25 W/m^2 at 42°C; Cramer & Jay, 2016).

2.1.1.2.2. Conduction

Thermal conduction is the rate of diffusive heat transfer between solid materials in direct contact with each other or through a non-moving gas or fluid (IUPS Thermal Commission, 2001). This heat transfer is dependent on the thermal gradient within the gas or liquid or between the surfaces and their thermal conductivity. For example, this can be within the

human body, where conduction is vital for transferring heat from the core to the periphery (i.e. skin) and between the skin and the ambient environment, where heat can be dissipated, if T_{sk} exceeds air temperature (IUPS Thermal Commission, 2001). However, heat dissipation by conduction is generally considered negligible, due to air's high thermal resistance. If skin is in direct contact with a much hotter or colder highly conductive surface, this can represent a major avenue or heat transfer (e.g. lying on the ground for a prolonged period or being immersed in water; Cramer & Jay, 2016, 2019; Romanovsky, 2007).

2.1.1.2.3. Convection

Convection is the rate of heat transfer between a body surface and the surrounding moving fluid, i.e. gas or liquid. This can be between different parts of the human body or between the human body surface (i.e. skin) and the ambient environment (IUPS Thermal Commission, 2001). Movement displaces the fluid layers closest to the skin, for example, which are closer to T_{sk} and replaces them with layers that are closer in temperature to the environment. Thus, convection is the amplification of conduction (Kerslake, 1972; Romanovsky, 2018). Again, this heat transfer is dependent on the temperature gradient between the skin and the surrounding environment and, as such, a greater T_{sk} will result in heat loss and a greater environmental temperature will result in heat gain. At rest, in still air, natural convection arises from the movement of air away from the skins surface, as it expands and warms (Kerslake, 1972). Heat transfer by convection can also be amplified by forced increased air movement, such as through wind, fans or body movement (IUPS Thermal Commission, 2001). This forced convection is also observed within the body, as blood flows from the core to the periphery, transporting heat for dissipation at the skin. At rest, in the majority of environmental conditions (20 to 40°C with both natural and forced convection), convective heat transfer represents the largest avenue of dry heat transfer with between -153 and 344 W or -79 and 177 W/m^2 lost (Cramer & Jay, 2016). Ultimately, the thermal gradients between the body's core and skin, and the ambient environment, dictate conductive and convective heat transfer.

2.1.1.3. Evaporation

Evaporation represents the change of state of a liquid to a vapour at a constant temperature (latent heat transfer) and the subsequent diffusion of this vapour into the surrounding air. Evaporative heat transfer represents heat loss occurring through this transition, as heat is required to facilitate it (Cramer & Jay, 2016). In humans, when sweat is evaporated from the skin surface, approximately 2.426 J/g of heat is lost (Wenger, 1972). The driving force for evaporation is the vapour pressure gradient present between the skin surface and the ambient

air (Cramer & Jay, 2016), but ultimately the amount of heat lost through evaporation is dependent on the latent heat of vaporisation (2.426 J/g at 30°C), convection at the skin surface and the quantity of sweat available (skin wettedness; Cramer & Jay, 2016). If the surrounding air temperature is greater than T_{sk}, evaporation is the only heat loss avenue available (Gagge & Gonzalez, 1996) and during exercise it is the primary avenue of heat dissipation, amounting to approximately 80% of all heat loss (Nielsen, 1938).

2.1.1.4. Heat storage

Heat storage is maintained if metabolic heat production and heat dissipation (through the above avenues) are balanced and is either positive (heat gain) or negative (heat loss) if there is an imbalance between these. Body heat storage ensues if the rate of heat production exceeds the rate of heat dissipation, due to insufficiency of these heat transfer avenues to meet heat loss demands or if dry heat transfer pathways (radiation, conduction and convection) are facilitating heat gain (Cramer & Jay, 2016; Kenny & Jay, 2011). Exercise induced high metabolic heat production enhances the need for heat dissipation and, therefore, in certain environmental conditions this leads to greater heat storage if it cannot be offset. Heat storage can also occur if high surrounding environmental temperatures exceed T_{sk}, limiting dry heat dissipation or promoting dry heat gain or if high ambient vapour pressure (i.e. high relative humidity [RH%]) of the surrounding environment limits evaporative heat dissipation. The rate of this heat transfer is governed by the physical properties of the skin (i.e. surface area, temperature and wettedness) and the environment (i.e. ambient temperatures, air flow and ambient vapour pressure; Gagge & Nishi, 2010). Prolonged heat storage inevitably results in increases to T_{core}, which if exceed the threshold of 40°C can have detrimental consequences to health.

2.1.2. Partitional calorimetry

Calorimetry is the measurement of heat transfer and is an important means by which to measure human heat balance (Cramer & Jay, 2019). This can be measured directly, using whole-body direct calorimeters which are insulated chambers that measure total heat dissipation from the individual inside (Kenny et al., 2017). While this is the gold standard method, it is extremely exclusive and largely inaccessible, which led to the development of other indirect methods (Cramer & Jay, 2019). Partitional calorimetry is a method by which to estimate human heat balance through the separate calculation of each heat transfer avenue based on the laws of thermodynamics which govern dry and latent heat transfer (Cramer &

Jay, 2019). These estimations can be made using relatively inexpensive equipment in either laboratory or field settings, as only a few measures are needed to make the majority of calculations. These include measurement of metabolic rate and external work to estimate heat production, measurement of the individual's height and mass, T_{sk}, body mass loss across the testing period, and measurement of the environmental conditions (ambient dry-bulb temperature [t_a], RH% and air velocity [V_{air}]) to estimate dry and evaporative heat transfer and heat storage, alongside other moderating variables (Cramer & Jay, 2019). Indeed, variables affecting these avenues of heat dissipation can also be estimated, such as the evaporative requirement for heat balance ($\dot{E}_{req}$), the maximum evaporative capacity of the environment ($\dot{E}_{max}$), skin wettedness and sweating efficiency (Cramer & Jay, 2019). See Chapter Three for all partitional calorimetry calculations used within the current thesis.

2.2. Thermoregulation

2.2.1. Physiological heat balance

The human body's ability to influence $\dot{H}_{prod}$ and heat transfer within the body and between the body and environment to maintain T_{core} is termed thermoregulation (IUPS Thermal Commission, 2001). Core temperature represents the temperature within the deep body tissues (e.g. brain, heart and abdominal viscera) and is approximately 37°C (Romanovsky, 2018). It is very stable and highly regulated, as a deviation of ± 3.5°C can cause various physiological impairments or even death (Moran & Mendal, 2002). Indeed, a T_{core} over 42°C (hyperthermia) is detrimental to cellular and organ function, leading to central nervous system and cardiovascular impairment, systemic inflammation and ultimately, multiple organ failure (Hifumi et al., 2018; Shapiro & Seidman, 1990). Such instances are preceded by symptoms of heat stroke, which are characterised by dizziness, disorientation, uncoordinated movement, fatigue and nausea (Yeo, 2004). Consequently, T_{core} must be maintained, despite varying external environmental conditions/temperatures and changing rates of internal $\dot{H}_{prod}$. This occurs through the sympathetic nervous system, where alterations to internal $\dot{H}_{prod}$, vasodilation and/or vasoconstriction and activation of eccrine sweat glands ensure thermal balance, predominantly by influencing avenues of heat transfers at the periphery (Parsons, 2014).

Surrounding the core is the shell, which encompasses the muscles, subcutaneous tissues and the skin (Lim et al., 2008). Largely, shell temperature represents that of the skin and is approximately 4°C lower than that of the core (Tansey & Johnson, 2015). However, it is thermally heterogenous, as there can be a wide variability in its temperature, due to exposure

to varying environmental conditions and subsequent changes to SkBF (Gisolfi & Mora, 2000). In hot environments this temperature difference between the core and skin decreases, as cutaneous blood flow (i.e. vasodilation) increases, transporting heat to the periphery for dissipation (Sawka & Wenger, 1988). In such conditions, blood flow is redirected to the skin surface and is the only controllable mechanism for transfer of heat from the core (González-Alonso, 2012). Through greater SkBF, enhanced conductive and convective heat exchange can take place (Charkoudian, 2010). As such the shell acts as a thermal buffer between the core and the environment, altering the temperature gradient between the two – through changes in SkBF – to either increase conservation or dissipation of heat in or from the core, respectively.

2.2.1.1. The influence of exercise

There are various environmental (ambient vapour pressure, ambient temperature; Kerslake, 1972) and physiological factors (dehydration, exercise; Mekjavic & Eiken, 2006) that affect thermal balance and heat transfer and, consequently the thermoeffector mechanisms of sweating and vasodilation. During prolonged or intense exercise - particularly in the heat - the ability to maintain thermal balance can become compromised (Sawka et al., 2011). Exercise increases $\dot{H}_{prod}$ at the working muscles (Gagge & Gonzalez, 1996), sometimes more than 20-fold (Sawka & Wenger, 1988). This subsequent increased muscle temperature leads to enhanced conductive and forced convective heat transfer between the muscles and circulating blood, transporting this heat around the body (Hardy, 1961). Core temperature consequently increases (Nielsen, 1938) and compensatory heat loss mechanisms (i.e. vasodilation and eccrine sweating) must be employed to meet the increased heat loss demands (Lim et al., 2008). The effector mechanisms of sweating and vasodilation, work concurrently to enhance the capacity to dissipate heat and may be activated up to the point of maximal vasodilation and sweat production if the conditions necessitate it (Benzinger, 1969).

2.2.1.2. The influence of environmental conditions

In hot environments and during exercise sweating is the primary mechanism that facilitates heat dissipation, with over 80% of heat loss occurring through evaporation (Gagge & Gonzalez, 1996; Nielsen, 1938). In such conditions, dry heat transfer is often insufficient to meet the heat loss demands and may not be a viable heat loss pathway at all if ambient temperature exceeds T_{sk} (Che Muhamed et al., 2016). Here, the temperature gradient will result in heat gain at the skins surface from the environment. Consequently, eccrine sweating

is the primary means by which heat balance is maintained during exercise and heat exposure, as it allows for evaporative cooling to offset $\dot{H}_{prod}$ (Marino et al., 2000; Sawka & Young, 2006). The efficiency of sweating and ambient vapour pressure, determine sweat evaporation off the skins surface (Gagge & Gonzalez, 1996; Parsons, 2007). There is a greater capacity to evaporatively cool in dry, hot environments compared to those with high humidity (Che Muhamed et al., 2016), as there is a superior $\dot{E}_{max}$, due to a larger vapour pressure gradient between the ambient air and skin's surface (Cramer & Jay, 2019). High sweat production and skin wettedness induce greater vapour pressure at the skin, which further raises this vapour pressure gradient and enhances the potential for evaporative heat loss (Kerslake, 1972). However, evaporation can only occur if ambient vapour pressure permits and, therefore, in conditions of high humidity, heat loss through this pathway is limited (Wenger, 1972). Thus, prolonged exercise in such conditions causes positive heat storage and thermal strain.

2.2.1.3. Exercising heat stress

Heat stress can be classified as either 'compensable' or 'uncompensable' (Kraning & Gonzalez, 1991). Compensable heat stress occurs when metabolic heat can be sufficiently dissipated to the environment, leading to balanced heat storage and maintenance of T_{core} (Cramer & Jay, 2016). For example, at exercise onset T_{core} will initially begin to increase, as $\dot{Q}$ is preferentially distributed to the working muscle. If exercise is of a low-moderate intensity, and environmental conditions are not too extreme, after a period of time a new steady-state is found and T_{core} will plateau, suggesting that a thermal equilibrium has been attained. Uncompensable heat stress occurs when the dissipation of metabolic heat to the environmental is insufficient, resulting in positive heat storage and rises in T_{core} that do not stabilise (Cheung et al., 2000; Cramer & Jay, 2016). The latter often occurs in hot and/or humid environmental conditions, where there is a reduced capacity for dry (conductive, convective, radiative) and evaporative heat transfer ($\dot{E}_{skin}$), respectively (Che Muhamed et al., 2016). The evaporative requirement for heat balance ($\dot{E}_{req}$), must not exceed the maximum evaporative capacity of the environment ($\dot{E}_{max}$), otherwise sufficient evaporative cooling cannot take place (Cramer & Jay, 2019). This can also occur if $\dot{H}_{prod}$ exceeds the maximum sweating capacity (Gagnon & Crandall, 2018). The transition from a thermally compensable to an uncompensable state is represented by an upward inflection in T_{core} (from its established steady-state value) and is termed the breakpoint of compensability (Ravanelli et al., 2018). This can be characterised in individuals using an 'inflection protocol' whereby either ambient dry-bulb temperature or relativity humidity incrementally are increased, until the critical point is reached at which heat dissipation can no longer compensate for metabolic heat production,

and T_{core} sharply rises. These are often used to assess thermal tolerance in differing populations (e.g. age and fitness), at differing exercise intensities and in differing clothing ensembles (Kenney et al., 1993; Kenney & Zeman, 2002; Kamon & Avellini, 1976; Kenney et al., 2020), as well as in individuals following interventions such as heat acclimation or exercise training (Ravanelli et al., 2018). However, there is limited investigation of the thermoregulatory efficacy of dietary supplements or other interventions designed to acutely affect thermoregulatory capacity.

Physical capacity and exercise performance are substantially impaired in hot and/or humid environmental conditions compared to those of lower temperature and vapour pressure (Galloway & Maughan, 1997; Hargreaves, 2008; Junge et al., 2016; Maughan et al., 2012), for several physiological reasons. This is largely due to increased T_{core} (i.e. thermal strain (González-Alonso et al., 1999), cardiovascular strain (Cheuvront et al., 2010) and an early onset of fatigue through reduction in central drive (Nybo, 2010). The cardiovascular system is placed under strain to support adequate $\dot{Q}$ to simultaneously and sufficiently perfuse both the cutaneous vasculature and all metabolically active tissues (González-Alonso, 2012; Rowell, 1974). If this cannot be appropriately balanced, this competition for blood flow ultimately may negatively impact maximal oxygen consumption (Arngrímsson et al., 2004; González-Alonso, 2012; Nybo et al., 2001), hastening a decrease in $\dot{Q}$ and blood pressure, resulting in reductions to blood flow, O_2 delivery, and uptake at the exercising muscle (González-Alonso & Calbet, 2003).

2.3. Thermoregulatory system

2.3.1. Thermoreceptors

In humans the thermoregulatory control centre is located in the preoptic anterior hypothalamus, with separate centres for controlling heat loss and heat gain mechanisms (Romanovsky, 2007). The hypothalamus uses sensory feedback from both central and peripheral thermoreceptors (specific sensory neurons) within the nervous system (Cheuvront & Haymes, 2001; Smith & Johnson, 2016) to detect thermal disturbances (Romanovsky, 2014). Central thermoreceptors are located in the brain (preoptic anterior hypothalamus), spinal cord and abdominal viscera, where they are primarily warm-sensitive (i.e. increase activity as temperature increases; Romanovsky, 2007). Peripheral thermoreceptors are located in the skin, and oral and urogenital mucosa, where they are primarily cold-sensitive (i.e. increase activity as temperature decreases). There are two types of skin thermoreceptors that are involved in thermoregulation; they respond to cold or warm stimuli and are positioned

in or immediately beneath the epidermis and slightly deeper in the dermis, respectively (Romanovsky, 2007). Both central and peripheral thermoreceptors transport afferent signals via temperature sensitive neurons to the preoptic anterior hypothalamus, where this sensory information is received, integrated and weighted (Smith & Johnson, 2016). Subsequently, efferent thermoregulatory pathways (autonomic nervous system) are either activated or inhibited (Charkoudian, 2016). These thermoreceptors and thermoeffectors, with their interconnecting afferent and efferent neural pathways, form feedback loops. There are two primary mechanisms that facilitate human heat dissipation: eccrine sweating and cutaneous vasodilation (thermoeffectors; Benzinger, 1969; Smith & Johnson, 2016).

2.3.2. Thermoeffectors

2.3.2.1. Eccrine sweating

Humans have three types of sweat glands: apocrine, apoeccrine and eccrine. Apocrine and apoeccrine sweat glands are not thought to have a thermoregulatory role (Sato et al., 1987), and, therefore, will not be detailed here. Eccrine sweat glands are the smallest but most common type of sweat gland, with approximately 2 to 4 million distributed across the human body (Weiner & Hellmann, 1960). They are found in both glabrous (palms, soles) and non-glabrous (hairy) skin, and open onto the skins surface (Kuno, 1938; Sato et al., 1989). They do not have a uniform spread across the entire body, with a greater density found on glabrous skin (250 to 550 glands/cm^2; Taylor & Machado-Moreira, 2013), compared to non-glabrous skin (2 to 5 fold less). While lesser in density the eccrine sweat glands on non-glabrous skin cover a larger surface area and are primarily responsible for thermoregulation (Baker et al., 2018). Eccrine sweat glands have a simple tubular epithelium, consisting of a secretary coil and a straight duct which opens out onto the skin surface (Sato, 1983). There are three main types of cells within the secretary coil; dark, clear and myoepithelial. The clear cells are primarily responsible for sweat secretion (Adrian et al., 1977; Costill, 1977; Sato & Sato, 1990). They contain basal infolding cell membranes, which contain sodium-potassium pumps and are responsible for driving this sweat secretion through sodium, potassium and chloride transport and Na-K-ATPase activity (Saga, 2002; Sato, 1993). The duct contains two cell layers; basal and luminal cells and its primary function is the reabsorption of Na and Cl ions from the secreted sweat (which is isotonic with blood plasma) as it passes through the duct (Sato et al., 1989). Consequently, sweat is fairly hypotonic (Adrian et al., 1977; Costill, 1977; Sato, 1993; Sato & Sato, 1990).

Whole-body sweat rate is determined by the number of active sweat glands and the rate of secretion per gland (Shibasaki et al., 2006). An increase in sweat rate is first facilitated by an increase in recruitment of sweat glands and followed by an increase in sweat output per gland (Buono & Connolly, 1992; Kondo et al., 1998; Kondo et al., 2001; Randall, 1946). Therefore, variations in sweat rates are largely attributed to the maximum sweat output achievable by a sweat glands and not the number of activated sweat glands (Sato & Dobson, 1970). In endurance trained and heat acclimated individuals, maximum sweat rates of 3 L/h have been observed, however the majority of individuals can achieve approximately 1.5 L/h (Taylor et al., 2008).

2.3.2.1.2. Central and peripheral mechanisms of thermal sweating

Thermal sweating is predominately mediated by sympathetic cholinergic stimulation (Nadel, Mitchell, et al., 1971). The preoptic anterior hypothalamus transmits sudomotor impulses via postganglionic nonmyelinated class C sympathetic fibres to eccrine sweat glands (Uno, 1977). Acetylcholine is released from the cholinergic nerve endings of these fibres, transverses the neuroglandular junction and binds to muscarinic receptors on the sweat gland - where it is hydrolysed by acetylcholinesterase - to stimulate sweating (Shibasaki et al., 2006). Sweat production is largely governed by the concentration of acetylcholine released from sudomotor nerve terminals and the speed of its breakdown into choline and acetate (Longmore et al., 1986). At low sweat rates acetylcholinesterase has a degree of influence on this output, but at high sweat rates its effects are negligible in comparison to acetylcholine release (Shibasaki & Crandall, 2010). Rises in T_{core} and T_{sk} and the subsequent increased central nervous system thermoefferent activity, induces acetylcholine release at the sudomotor junction (Shibasaki & Crandall, 2010). Integration of both T_{core} and T_{sk} have been demonstrated to control the sweating response in humans (Nadel et al., 1971a, b). Indeed, sweating is directly related to T_{core} when T_{sk} is controlled, and also T_{sk} when T_{core} is controlled (Nadel et al., 1971a, b).

As well as being affected by T_{core} and T_{sk}, sweating can also be modulated by the local temperature of the site being assessed (van Beaumont & Bullard, 1965). Indeed, at fixed T_{core} and T_{sk}, warming of a measured site results in increased local sudomotor function (Nadel et al. 1971a, b). This may be due to the direct effect of temperature on the sweat gland or the concomitant effects on cutaneous vasodilation (Johnson et al., 2011). However, Wingo et al. (2010) established that local heating increased sweat rates when levels of SkBF were pharmacologically maintained, indicating that local T_{sk} effects sweating independent of SkBF changes. This may be due to a direct effect of temperature on acetylcholine release at the sweat gland or the sensitivity of cholinergic receptors (MacIntyre et al., 1968; Ogawa, 1970).

The characterisation of the effects T_{core} or T_{sk} have on thermoeffector output (e.g. sweating response), and potential modulating variables requires one to be plotted as a function of the other. Indeed, the temperature at which the thermoeffector output begins to rise is known as the onset threshold and the subsequent slope of the relationship between temperature and thermoeffector output is known as the thermosensitivity (Hammel, 1968; Cheuvront et al., 2009). These relationships are considered indicators of central (onset threshold) and peripheral or local (thermosensitivity) thermoregulatory control (Ravanelli et al., 2019; Nadel et al., 1971b, 1974). Therefore, any alterations in either the onset threshold or the thermosensitivity will likely be representative of a central or peripheral effect, respectively. There are two important aspects to thermoregulatory sweating: onset (T_{core} threshold) and sensitivity (slope of the relationship between sweat rate and T_{core} change) of the sweating response to hyperthermia (Armstrong & Maresh, 1998). Changes to the sweating set-point are thought to be central (hypothalamic), whereas changes to sensitivity are thought to be peripheral (sweat gland; Nadel, 1979).

2.3.2.1.3. Non-thermal controllers of sweating

2.3.2.1.3.1. Adrenergic mechanisms

While cholinergic innervation of sweat glands is the primary established pathway (Chalmers & Keele, 1952), adrenergic mechanisms also appear to be involved (Donadio et al., 2006; Warndorff & Neefs, 1971). Adrenergic mechanisms are, therefore, thought to be a non-thermal stimuli for the sweating response (Robertshaw, 1977; Nagazato et al., 2004), with sweat glands responding to beta and alpha-adrenergic agonists; however, their contribution to sweating is relatively minor in comparison (Sato & Sato, 1981, 1984), as exogenous administration of adrenergic agents stimulates sweating to a much lesser degree than acetylcholine administration (Baum et al., 1976; Buono et al., 1992; Lee et al., 2014; Libert et al., 1988; Allen & Roddie, 1972; Wolf & Maibach, 1974; Sato & Sato, 1981; Shibasaki et al., 2001). Adrenergic neurons are found in relative proximity to eccrine sweat glands (Uno, 1977; Donadio et al., 2006), although these, alongside adrenergic receptors are fewer in quantity and density than cholinergic neurons and receptors (M3). These appear to have little influence on thermoregulatory sweating as sweat production during exercise (Buono et al., 2011) and following heat acclimation (Martinez et al., 2012) are unaffected by beta-adrenergic blockade. Therefore, sweating is primarily stimulated by acetylcholine release (Dale & Feldberg, 1934) from cholinergic nerves and its binding to muscarinic (M3 subtype) receptors on eccrine sweat glands (Grant et al., 1991; Torres et al., 1991).

2.3.2.1.3.2. Exercise

Exercise related non-thermal stimuli are also thought to mediate the sweating response (Shibasaki & Crandall, 2010; Shibasaki et al., 2003). At the onset of both dynamic and isometric exercise, sweating occurs almost immediately (1.5 to 2 s; Van Beaumont & Bullard, 1963; Yanagimoto et al., 2003), and before any substantial rises in either T_{core} or T_{sk}. Alongside the observation that intermittent exercise causes rapid spikes in sweat rates following changes in workload, this suggests that exercise somehow independently modulates the sweating response. Augmentation of central command during isometric handgrip (IHG) exercise has been shown to further increase sweat rate, indicating a potential role in stimulating sweating (Shibasaki et al., 2003). Stimulus from baroreceptors also influences the sweating response. Indeed, attenuation of the reduction in post-exercise mean arterial pressure (MAP), allows sweat rate to remain elevated (Journeay et al., 2004; Journeay et al., 2005; Kenny & Jay, 2013). Further, sweat rate assessed during post-exercise ischaemia following isometric exercise remained elevated, and only returned to baseline after the cessation of ischaemia (Crandall et al., 1998; Kondo et al., 1999; Shibasaki et al., 2001). This is suggestive of muscle metaboreceptor involvement in modulating sweating during exercise. To expand on this Shibasaki et al. (2001) conducted a study where during post-exercise ischaemia, blood pressure was returned to baseline through administration of intravenous sodium nitroprusside. As elevated blood pressure is thought to contribute to the sweating response, due to baroreceptor unloading, this design isolated the effect of muscle metaboreceptors. Sweating rate still remained high, despite blood pressure decreasing, indicating muscle metaboreceptor involvement (Shibasaki et al., 2001).

2.3.2.1.3.3. Dehydration (hypohydration)/hyperosmolality

Dehydration has been demonstrated to delay and/or reduce the sweating response (Montain et al., 1995; Sawka et al., 1985). This often occurs with periods of high sweat rates during exercise, where fluid losses cannot be sufficiently replaced by fluid intake (Sawka et al., 1984). Hyperosmolality and hypovolaemia occur with exercise induced dehydration as sweat is hypotonic to blood plasma and plasma volume is reduced with the enhanced fluid loss through sweat (Costill et al., 1974; Costill et al., 1976). Hyperosmolality raises the onset threshold (i.e. T_{core} - sweating threshold) for sweating (Fortney et al., 1984; Ito et al., 2005; Libert et al., 1988; Lynn et al., 2012; Sawka et al., 1985), and hypovolemia appears to reduce thermosensitivity (Fortney et al., 1981), though this has been observed inconsistently (Gagnon & Crandall, 2018; Libert et al., 1988; Montain et al., 1995; Fortney et al., 1984). Further, independent of plasma volume (i.e. hypovolaemia), hyperosmolality appears to reduce sweating, with the

impairment to sweating often proportional to the degree of hypohydration (Sawka et al., 1985; Montain et al., 1995). This attenuates sweating likely through the action of baroreceptors (hypovolemia) and/or osmoreceptors (hyperosmolality). However, it appears that baroreceptor unloading is only responsible for modulating sweating during- and post-dynamic exercise (Fortney et al., 1981; Mack et al., 1995, 2001; Journeay et al., 2004, 2007; McInnis et al., 2006; Kenny & Gagnon, 2010; Paull et al., 2016), and not during passive heat exposure (Solack et al., 1985; Vissing et al., 1994; Kenny et al., 2010; Binder et al., 2012; Lynn et al., 2012; Schlader et al., 2015a).

2.3.2.1.3.4. Biophysical factors

Difference in sweat rates have been demonstrated between men and women, which is largely due to confounding physical characteristics and other factors independent of sex. Men have a greater maximal sweating rate and cholinergic responsiveness than women (Gagnon & Crandall, 2018; Gagnon et al., 2013; Gagnon & Kenny, 2012; Inoue et al., 2014). However, studies which have matched men and women for body mass, surface area and overall $\dot{H}_{prod}$ have shown little difference in whole-body sweat production (Gagnon & Crandall, 2018; Gagnon et al., 2013; Gagnon & Kenny, 2011, 2012). Differences were only observed when there was a high evaporative requirement for heat balance (35 to 40°C; 12% RH; 300 to 500 W/m^2), in which the greater maximal sweat rate of males was necessary (Gagnon & Crandall, 2018; Gagnon et al., 2013; Gagnon & Kenny, 2011, 2012). Due to their general lower body surface women generally have a higher sweat gland density (Buono & Sjoholm, 1988) and, therefore, lower sweat output per gland (Gagnon & Kenny, 2012; Inoue et al., 2014). This explains why in some studies women appear to have a lower sweat rate (Gagnon & Kenny, 2012; Inoue et al., 2014). The higher sweat rate observed in men is generally due to greater body mass and consequently greater $\dot{H}_{prod}$ at a given $\dot{V}O_2$/intensity (Avellini et al., 1980; Havenith & van Middendorp, 1990; Notley et al., 2017; Shapiro et al., 1980; Smith & Havenith, 2012b).

2.3.2.1.4. Drivers of sweating

It has long been thought that the T_{core} and T_{sk} attained during exercising heat stress directly determine the thermal sweating response (Belding & Hertig, 1962; Nielsen & Nielsen, 1965; Benzinger, 1969; Nadel, 1979). However, more recently it has been established that $\dot{H}_{prod}$ and $\dot{E}_{req}$ are the primary drivers of the steady-state thermal sweating response (Cramer & Jay, 2014, 2016; Gagnon et al., 2013; Chapter Six). Sweat production and $\dot{E}_{req}$ share a close

relationship, with sweat rates greater in hotter conditions (both exercising and passive), where greater evaporation is required to maintain heat balance ($\dot{E}_{req}$). Given that the primary function of the thermoregulatory system is to achieve thermal balance and control T_{core} (Nielsen, 1938), it follows that the sweating response should be dictated by the requirement for heat balance (e.g. $\dot{E}_{req}$). Gagnon et al. (2013) measured whole-body sweat rate (WBSR) at various required evaporations for heat balance through the manipulation of exercise intensity (i.e. $\dot{H}_{prod}$) and ambient temperature. At a fixed $\dot{H}_{prod}$, increasing ambient temperature resulted in a linear increase in WBSR. This was due to the reduced capacity for dry heat losses and subsequent greater need for evaporation to maintain thermal balance. Conversely, at a fixed ambient temperature, increasing $\dot{H}_{prod}$, drove a greater WBSR. Collectively this demonstrates an association between sweat production and $\dot{E}_{req}$, with approximately 95% of variance in WBSR explained by the latter. Similar steady-state sweating responses have been observed across a range of absolute T_{core} and T_{sk} in conditions that evoked the same $\dot{E}_{req}$ (Ravanelli et al., 2020). There is also a positive relationship between whole-body sweating and $\dot{H}_{prod}$ (Hospers et al., 2020), which follows as the net difference between $\dot{H}_{prod}$ and dry heat exchange is $\dot{E}_{req}$ (Cramer & Jay, 2019). Both $\dot{H}_{prod}$ and $\dot{E}_{req}$ are modifiable variables responsible for driving the rate of evaporative cooling and can be estimated using partitional calorimetry in a controlled laboratory setting (Cramer & Jay, 2019). Metabolic heat production and $\dot{E}_{req}$ are often controlled during experimental studies in order to control the thermal sweating response. Any notable changes in sweating can then be established, such as in response to an intervention, for example. Therefore, appropriate characterisation of the multiple aspects of the sweating response with these drivers is necessary to be able to decipher any changes within them. However, the relationship between $\dot{H}_{prod}/\dot{E}_{req}$ and other aspects of the sweating response have not been established, such as SGA. Furthermore, the reliability of controlling $\dot{H}_{prod}$ and $\dot{E}_{req}$ has not been evaluated, which is necessary to be able to reliably control the thermal sweating response and, consequently, assess changes too it.

2.3.2.1.5. Variability in sweating and thermoregulatory responses

There is substantial inter-individual variability in thermoregulatory capacity, even among healthy individuals (Cramer & Jay, 2015; Lind et al., 1970), with many demonstrating an inferior ability to activate thermoregulatory defences and, thus, have a greater risk of heat related illness. There are several physiological and biophysical factors that can affect the ability to thermoregulate. These include body size (e.g. mass, fat mass and body surface area [BSA]), aerobic fitness (i.e. $\dot{V}O_{2max}$) and heat acclimation status (Cramer & Jay, 2016). Fairly recently, multiple studies have demonstrated that the differences in sweating and T_{core}

responses seen in individuals with varying body sizes and $\dot{V}O_{2max}$ values are largely eliminated when factors such as $\dot{H}_{prod}$ and $\dot{E}_{req}$ are controlled for these variables (e.g. when $\dot{H}_{prod}$ and $\dot{E}_{req}$ are expressed in W/m^2, for example; Cramer et al., 2012; Havenith et al., 1998). Controlling exercise by $\dot{H}_{prod}$ and by BSA (m^2) in stable environmental conditions, and subsequently controlling $\dot{E}_{req}$, eliminates 54 to 71% of individual variability in T_{core} and thermal sweating responses to exercise in a compensable environment (Cramer & Jay 2015). Investigation into thermoregulatory capacity during exercising heat stress requires valid and reliable measurements of thermal sweating and its determining factors (i.e. $\dot{H}_{prod}$ and $\dot{E}_{req}$).

2.3.2.2. Vascular function

2.3.2.2.1. Vasodilation

Alterations to cutaneous vascular tone is achieved through adjustments in two different nerve types, non-adrenergic vasodilator and adrenergic vasoconstrictor nerves, which are under autonomic control (Kellogg, 2006; Smith & Johnson, 2016). Cutaneous vasodilation during heat stress is controlled by sympathetic neurogenic mechanisms, alongside local mechanisms and are partially facilitated by withdrawal of vasoconstrictor tone, but primarily by activation of the active sympathetic vasodilator system (>80 to 90%; Charkoudian, 2003; Johnson et al., 2011). In thermoneutral conditions, there is a baseline level of vasoconstrictive tone, with the initial response to heat stress being passive vasodilation via sympathetic nervous system withdrawal (Johnson, 2010; Johnson et al., 1995). As vasoconstriction is adrenergic, it is activated through the release of norepinephrine and cotransmitters by adrenergic nerves (Johnson & Kellogg, 2010; Johnson et al., 2014). Therefore, withdrawal is initiated through a reduction in vasoconstrictor nerve activity (Smith & Johnson, 2016). If this is insufficient to meet heat loss demands, then the vasodilatory system is activated to enhance SkBF further (Johnson et al., 2011). Active vasodilation occurs in response to acetylcholine and other cotransmitters (e.g. vasoactive intestinal polypeptide; Bennett et al., 2003) release from sympathetic cholinergic nerves (Edholm et al., 1957). These cotransmitters act on pituitary adenylate cyclase-activating peptide 1 receptor (PAC1) and vasoactive intestinal peptide receptors (VPAC) receptors on the endothelial cells of the vascular smooth muscle to produce predominantly nitric oxide (NO). Nitric oxide is an important signalling molecule for active cutaneous vasodilation (Kellogg et al., 1998; Shastry et al., 1998). Indeed, heat stress induced vasodilatory responses are reduced by approximately 30% following the blockade of NO synthase enzymes. Hydrogen sulphide (H_2S) is another endogenous gasotransmitter, with vaso-active properties (Zhao et al., 2001; Yang et al., 2008). It is synthesised by the enzymes

cystathionine γ-lyase (CSE) and 3-mercaptopyruvate sulphurtransferase (3-MST) in response to cholinergic stimulation (Mustafa et al., 2011). Indeed, CSE knockout mice demonstrate reduced H_2S synthesis, leading to impaired endothelium-dependant vasodilation and hyperpolarisation (Yang et al., 2008; Mustafa et al., 2011). Further, acetylcholine dependent vasodilation was blunted in rat aorta with the inhibition of H_2S synthesis (Paredes et al., 2012). Hydrogen sulphide appears to regulate vascular tone (Siebert et al., 2008; Yang et al., 2008), through ATP-activated potassium channels (Maia et al., 2014; Ulusoy et al., 2017). Total SkBF is approximately 250 to 300 mL/min in thermoneutral conditions; however, there is a large potential variability depending on the environmental conditions. For example, SkBF can range from nearly zero (in extreme cold) up to 6 to 8 L/min (in extreme heat; Charkoudian, 2003; Johnson & Kellogg, 2010; Johnson et al., 2011; Rowell, 1974). In the forearm, maximal SkBF is suggested to be achieved at a T_{sk} of 42°C (Taylor et al., 1984). To attain this, HR and $\dot{Q}$ must increase and blood flow must be redirected from other vascular beds such as renal and splanchnic (Charkoudian, 2003). During exercise, this puts a large strain on $\dot{Q}$ to supply both the cutaneous vasculature with sufficient blood flow, as well as the active muscles (Simmons et al., 2011).

2.3.2.3. Relationship between sweating and vasodilation

Skin blood flow has an important role in facilitating sweating and evaporative heat dissipation, as it provides the fluid for sweat production and also the heat to be evaporated (Smith & Johnson, 2016). Both can be activated through cholinergic stimulation (Kellogg et al., 1995), but despite this they do not always appear to have the same onset thresholds (Shibasaki et al., 2002). There is no consistent overlap of SkBF and sweat production across the body surface, as a large variation in sweating is found in varying body regions, but this is not the case for SkBF (Smith et al., 2013a; Smith et al., 2013b). Nevertheless, an association between sweating and SkBF has been established (Van Beaumont & Bullard, 1965; Brengelmann et al., 1973; Nadel et al., 1971a, b), suggesting a function relationship between the two (Wong & Hollowed, 2017). A requirement of sufficient SkBF has been established for a profuse sweating response (Wingo et al., 2010). However, findings by Ravanelli et al. (2017) demonstrate that increases in SkBF are not a prerequisite for increases in LSR. While this may be the case, attenuation of SkBF through arterial occlusion (Collins et al., 1959; MacIntyre et al., 1968) or pharmacological blockade (Wingo et al., 2010) reduces the sweating response during heat stress, suggesting a requirement of SkBF for sweating.

2.4. Modifiable factors

An individual's thermoregulatory capacity can be acutely or chronically enhanced. There are three main factors which can be modified in order to achieve this; lowered metabolic heat production, enhanced vasodilation (i.e. dry heat loss) and increased sweating (i.e. evaporative heat loss; Benzinger et al., 1961b; Gagge & Gonzalez, 1996; Wendt et al., 2007). As such, the ability to activate thermoregulatory defences and tolerate hot environmental conditions can be improved. Sweating is the primary manipulable pathway in which this can occur (Périard et al., 2016; Ravanelli et al., 2018; Wenger, 1972). Indeed, endurance training and heat acclimation regimes are both interventions which have demonstrated the capacity to accelerate sweating onset and increase sweat rate. They have also been shown to lower the oxygen cost of exercise and resting T_{core}, and increase plasma volume and SkBF (Lorenzo et al., 2010; Périard et al., 2016; Poirier et al., 2016; Ravanelli et al., 2018; Rivas et al., 2017). Overall, these physiological adaptations augment avenues of heat transfer, control $\dot{H}_{prod}$ and, aid in the maintenance of thermal equilibrium during heat stress.

2.4.1. Endurance training and heat acclimation

The sweating response is enhanced with interventions such as aerobic/endurance training (Buono & Sjoholm, 1988; Buono et al., 1991; Greenleaf et al., 1972; Inoue et al., 1999) and heat acclimation (Allan & Wilson, 1971; Kirby & Convertino, 1986; Pandolf et al., 1988), where enhanced sweating and improvements to the onset threshold and thermosensitivity of the response are observed (Baum et al., 1976; Buono et al., 1992; Lee et al., 2014; Nadel et al., 1974; Pandolf et al., 1988; Roberts et al., 1977; Taylor, 2014). Through the augmentation of thermal sweating, the efficiency of evaporative heat transfer can also be improved (Ravanelli et al., 2018). Consequently, the ability to tolerate hot environmental conditions can be modified (Périard et al., 2021). Indeed, endurance training improves the sweat gland responsiveness to cholinergic agonists (Buono and Sjoholm, 1988; Buono et al., 1992; Wilson et al., 2010) and has also been shown to lead to activation of a greater number of sweat glands and skin wettedness (Ravanelli et al., 2018). This likely explains why these individuals can attain greater maximal sweat rates and, consequently, improved evaporative heat dissipation (Lamarche et al., 2018).

Similarly, heat acclimation augments the sweat response, with greater maximal values, an earlier onset threshold and greater thermosensitivity of the sweat rate response (Wyndham, 1967). Indeed, improvements to SGA, onset threshold and thermosensitivity have repeatedly be observed following 8 to 14 days of heat exposure (Klous et al., 2020; Lorenzo et al., 2010;

Poirier et al., 2016; Ravanelli et al., 2018). Heat acclimation is the process by which repeated exposure to artificially induced heat stress induces various physiological adaptations (IUPS Thermal Commission, 2001). The thermoregulatory adaptations brought on by heat acclimation are greater than by endurance training alone (Ravanelli et al., 2018). The greater sweating rates observed are at least partially through a central adaptation, as sweating across the whole-body surface is enhanced (Patterson et al., 2004). This results in a greater overall skin wettedness (Candas et al., 1979; Ravanelli et al., 2018), which allows for a greater capacity for evaporative heat transfer if the environmental conditions permit (Poirier et al., 2015). The reduced onset threshold is also representative of a central mechanism (Fox et al., 1963; Nadel et al., 1974; Roberts et al., 1977; Armstrong & Kenney, 1993; Poirier et al., 2015, 2016), but it parallels the heat acclimation induced reduction in resting T_{core} (Patterson et al., 2004). This puts into question whether heat acclimation actually changes the T_{core} - sweating onset threshold, as the absolute change in T_{core} required to initiate sweating remains the same (Patterson et al., 2004; Poirier et al., 2015, 2016). Nevertheless, a peripheral mechanism has also been ascribed to the improved sweating responses observed. Similarly, as with endurance training, heat acclimation improves the responsiveness of eccrine sweat glands to cholinergic agonists (Inoue et al., 1999; Buono et al., 2008, 2009; Lorenzo et al., 2010). Cholinergic sensitivity and sweat gland output are correlated with gland size (Sato & Sato, 1983) and, therefore, a structural adaptation to eccrine glands may be responsible for improved thermosensitivity (Fox et al., 1963; Nadel et al., 1974; Roberts et al., 1977; Armstrong & Kenney, 1993; Patterson et al., 2004; Poirier et al., 2015, 2016). This is thought to be brought about by repeated sweat gland activity during the heat acclimation process (Buono et al., 2009). Ravanelli et al. (2018) demonstrated an improved heat tolerability with endurance training and heat acclimation. The reported augmentation of the sweating response delayed the breakpoint of compensability, signifying an enhanced ability to tolerate heat stress.

2.4.2. Dietary supplementation

The effects endurance training and heat acclimation have on the sweating and vascular response demonstrate that thermoregulatory capacity can be enhanced. There is evidence to suggest that more acute interventions such as the use of dietary supplements may be able to offer similar thermoregulatory benefits (Jardine et al., 2023; Twycross-Lewis et al., 2016). For example, a recent review recognised that creatine confers thermoregulatory benefits during exercise in the heat due to its effects on fluid balance (Twycross-Lewis et al., 2016). Similarly, pre-exercise hyperhydration with glycerol and/or creatine supplementation has been demonstrated to lower end T_{core} in hot conditions following exercise (Jardine et al., 2023). Additionally, dietary nitrate can be supplemented to improve NO bioavailability (Lundberg et

al., 2008; Moncada & Higgs, 1991). This may have both direct and indirect effects on vascular and eccrine sweat gland function (Fujii et al., 2016; Stapleton et al., 2014a). Furthermore, anti-oxidants, may offer protection of NO against oxidative destruction, thereby maintaining its bioavailability and potentially improving thermoregulation (Ignarro et al., 2006). Finally, taurine supplementation has been shown to improve thermal sweating during exercise in the heat and subsequently reduce end T_{core} compared to placebo (Page et al., 2019). Indeed, many of the biological roles ascribed to taurine, have the potential to provide a thermoregulatory advantage during heat exposure, based upon the relationship to avenues of heat loss (Huxtable, 1992). However, only two studies have investigated some aspects of thermoregulation in response to taurine ingestion and, therefore, a much more thorough evaluation of its potential impact on thermoregulatory responses is warranted. Collectively, it is clear that dietary supplements potentially have the capacity to influence thermoregulatory process and, ultimately, affect thermal balance; however, further research is needed to corroborate these findings, expand upon them, and provide preliminary mechanistic insights.

2.5. Taurine

Taurine, a sulphur containing amino acid, is found in the majority of animal products such as eggs, meats and seafood (Abebe & Mozaffari, 2011; Laidlaw et al., 1990). These dietary sources are the primary means by which mammalian taurine is obtained. However, it can also be endogenously synthesised from the semi-essential amino acid cysteine in a three-step process (which is in turn synthesised from methionine; Bin et al., 2017). Taurine is the most abundant free amino acid in mammalian tissues and is found in particularly high concentrations in excitable energy-consuming tissues such as the brain, heart and oxidative skeletal muscle (Jacobson & Smith, 1968; Huxtable 1992). It is involved in numerous biological processes, such as intracellular osmoregulation, anti-inflammation and regulation of mitochondrial metabolism, calcium homeostasis and oxidative stress (Huxtable 1992; Schaffer et al., 2010).

When orally supplemented in thermoneutral conditions, taurine has been demonstrated to improve endurance exercise performance (Balshaw et al., 2013; Waldron et al., 2019; Waldron et al., 2018a; Zhang et al., 2004). These observed ergogenic effects are similar in magnitude to those seen following endurance training or heat acclimation (Waldron et al., 2021). Mechanistically, performance improvements may be related to sarcoplasmic reticulum Ca^{+} handling (Dutka et al., 2014; Hamilton et al., 2006), anti-oxidative effects (Hansen et al., 2006; Hansen et al., 2010; Jong et al., 2021; Schaffer et al., 2022; Zhang et al., 2004) and/or alterations in substrate utilisation (Rutherford et al., 2010; Simmonds et al., 2022). These

adaptations are all features of the endurance trained phenotype (Lima-Silva et al. 2010). Indeed, taurine appears to regulate mitochondrial function, through its roles as an anti-oxidant, acting as a mitochondrial matrix buffer (Hansen et al., 2006; Hansen et al., 2009). It also improves skeletal muscle contractility (Pierno et al., 1996; Seidel et al., 2019) and is associated with enhanced oxidative metabolism (Hansen et al., 2010) and muscle function (Seidel et al., 2019). Indeed, in the exercising human, changes in metabolic responses have been observed with taurine supplementation, alongside increased maximal oxygen consumption (Zhang et al., 2004; Balshaw et al., 2013). It appears that during exercise (metabolic work) taurine may shift metabolism towards oxidative pathways, improving exercise efficiency and that of ATP synthesis in the muscle (Hansen et al., 2006; Figure 2.1.f).

Taurine appears to have a key role in cardiac function (Schaffer et al., 2010), largely through regulation of sarcoplasmic reticulum Ca^{+} handling and Ca^{+} sensitivity. This has been observed in animal cardiac muscle cells, where taurine enhances contractile function (Schaffer et al., 2010) and has a positive inotropic effect (Satoh & Sperelakis, 1998). Additionally, ingestion of taurine as part of an energy drink, has previously increased SV (Baum & Weiss, 2001); however, changes in HR have not been observed during exercise in the heat following taurine ingestion (Page et al., 2019; Figure 2.1.e). A characteristic of the heat acclimated phenotype is increased SV and $\dot{Q}$ (Périard et al., 2016), which most likely reflect an enhanced cardiac stability or efficiency (Horowitz, 2002). Considering taurine's potential effects, it is feasible that it could provide similar benefits during periods of cardiovascular strain (i.e. heat stress, however, this requires further evaluation to elucidate). The performance enhancing effect of taurine supplementation (50 mg/kg) was heightened when administered during exercise in the heat, prolonging time to exhaustion by ~10% (Page et al., 2019) and ~15% (Yu et al., 2024). Further, Page et al. (2019) also observed an enhanced sweating onset and rate (~12.7%; Figure 2.1.a). End T_{core} was significantly reduced (38.1°C *vs* 38.°C [Page et al., 2019]; 38.2°C *vs* 38.4°C [Yu, et al., 2024) in both studies (Figure 2.1.d), suggesting taurine does have a thermoregulatory role in humans during exercising heat stress.

2.5.1. Vascular function

Additional physiological functions of taurine may also be beneficial during heat exposure, such as cellular osmoregulation (Cuisinier et al., 2002) and its vasoactive properties (Maia et al., 2014; Sun et al., 2016; Ulusoy et al., 2017). Indeed, previous *in-vitro* studies have established taurine's ability to elicit peripheral vaso-relaxation in macro-vessels (Ulusoy et al., 2017; Yildiz & Ulusoy, 2022; Figure 2.1.b). Both endothelium-independent and dependant mechanism have be ascribed to these effects and they include reduction in Ca^{2+} influx and release

(Dawson Jr et al., 2000; Franconi et al., 1982; Harada et al., 2004; Li et al., 2009), anti-oxidation (Hagar et al., 2006; Leão et al., 2019; Maia et al., 2014; Rahman et al., 2011), improved NO bioavailability (Maia et al., 2014; Rahman et al., 2011), elevation of plasma H_2S concentrations (Sun et al., 2016) and K^+ channel opening action (Liu et al., 2009; Ulusoy et al., 2017). Taurine's effects on endothelium-dependant and independent vasodilation have also been demonstrated on humans, *in-vivo* (Sun et al., 2016). Improvements in both endothelium-independent (4.4% increase in nitro-glycerin mediated dilation) and dependant vasodilation (3.2% increase in flow-mediated dilation [FMD]) in pre-hypertensive patients has also been observed (Sun et al., 2016; Figure 2.1.b). This is likely attributed to improvements in NO bioavailability (Palmer et al., 1987) and increased synthesis of the signalling molecule H_2S (Liang et al., 2011; Sun et al., 2016). In this clinical study, taurine supplementation was shown to increase circulating concentrations of H_2S and its precursors CSE and cystathionine-β-synthase (CBS; Sun et al., 2016), suggesting it may be responsible for the observed effects.

Taurine may also affect vascular tone directly, as within the arterioles of vascular smooth muscle are many taurine transporters (Taut; Liao et al., 2007). This may be through its effects on calcium handling and K^+ channels (Li et al., 1996; Liu et al., 2009; Ristori & Verdetti, 1991; Ulusoy et al., 2017), as endogenous taurine has been shown to modulate Ca^{2+} and K^+ channel function (Franconi et al., 1982). *In-vitro* studies of rodent vessels have reported that at high intracellular Ca^{2+} concentrations associated with hypoxic conditions, taurine inhibited Ca^{2+} channel function to induce vasodilation, with the opposite effect reported following low intracellular Ca^{2+} concentrations (Yildiz & Ulusoy, 2022). Taurine has repeatedly been shown to reduce resting blood pressure in human trials in thermoneutral conditions (Waldron et al., 2018b; Figure 2.1.c). These participants, however, are often hypertensive or suffering from a range of other comorbidities (Sun et al., 2016; Waldron et al., 2018b). Nevertheless, chronic taurine administration lowered systolic blood pressure by approximately 3 mmHg (Waldron et al., 2018b). Collectively, this was attributed to a vasodilatory role (Sun et al., 2016; Waldron et al., 2018b), which may be beneficial during heat exposure to facilitate dry and evaporative heat transfer, however, this is yet to be investigated *in-vivo*.

2.5.2. Sudomotor function

There is currently limited understanding of the mechanistic explanation for taurine's effects on the sweating response during heat stress. However, the earlier onset of sweating is indicative of a centrally-mediated mechanism, potentially related to its role as a neuromodulator (Hussy et al., 2000; Jia et al., 2008). Indeed, taurine is a neuromodulator, acting as a gamma-aminobutyric acid (GABA) receptor agonist, where it functions to protect neurons from toxicity

by modulating thalamic network activity under conditions of homeostatic derangement, which are associated with severe pathological conditions and may be extended to transient states of heat stress (Jia et al., 2008). Animal studies have identified that during thermal strain taurine and GABA are released from some hypothalamic cells into the cerebrospinal fluid and that that is concomitant with T_{core} reductions (Frosini et al., 2000). Theoretically, increased plasma taurine will be available to cross the blood-brain barrier via TauT following oral supplementation (Kang, 2000), and act on hypothalamic regions of the brain, potentially interacting with a specific taurine binding site (i.e. putative Taurinergic pathway) or GABA receptors (Frosini et al., 2003; Queva et al., 2003). It is possible that during heat stress this cryogenic pathway (Frosini et al., 2003; Elhussiny et al., 2021) may translate to enhanced sudomotor function, as the major effector response.

Another potential mechanism for the increased sweating observed is through taurine antagonism of antidiuretic hormone (ADH) or arginine vasopressin (AVP). Vasopressin is an antidiuretic hormone produced in the supraoptic nucleus of the hypothalamus and released by the posterior pituitary gland in response to plasma hyperosmolality (Cunningham & Sawchenko, 1991; Bourque et al., 1994; Richard & Bourque, 1995). At the kidney, ADH acts on vasopressin subtype 2 receptors (V2 receptors), promoting fluid and sodium reabsorption to maintain fluid homeostasis (Knepper et al., 1999). Taurine's release from the hypothalamus in response to plasma hypoosmolality is suggested to exert an inhibitory effect on ADH secretion (Deleuze et al., 1998; Hussy et al., 1997; Miyata et al., 1997), thereby promoting increased fluid loss. Supporting this, taurine-depleted rats have shown increased baseline plasma ADH concentrations and altered renal excretory function, including increased urine osmolality (Mozaffari & Schaffer, 2001). Subsequent repletion of taurine partially reversed these induced effects, suggesting that taurine has a role in the maintenance of body fluid homeostasis through an ADH-dependant mechanism (Mozaffari & Schaffer 2001). Sweat glands share many similarities with the kidneys and it has been suggested that ADH may facilitate fluid reabsorption at the sweat gland, as it does in the kidneys (Agu, 2017). As such, exogenous taurine supplementation may supress the release of ADH and attenuate water reabsorption at the sweat gland, leading to greater fluid loss. In the rat model subcutaneous injection of ADH reduced initial sweat rate by 50%, suggesting a role for ADH in regulating the sweating response (Quatrale & Spier, 1970). Nevertheless, several studies both augmenting and suppressing ADH have observed no significant change in sweat rate during exercise or heat exposure (Pearcy et al., 1956; Senay & Van Beaumont, 1969; Ratner & Dobson, 1964; Gibinski et al., 1979; Tausisig & Braunstein, 1973; Ladell & Whitcher, 1960; Allen & Roddie, 1974; Hew-Butler et al., 2014).

One of taurine's primary biological functions is as an osmolyte (Cuisinier et al., 2002; Huxtable, 1992), with many tissues harbouring TauT (Baliou et al., 2020; Han et al., 2000; Han et al., 2006; Ito et al., 2010). Consequently, taurine has potential to affect fluid regulation at the cellular and organ level. Indeed, during exercise taurine is actively extruded from skeletal muscle cells (Graham et al., 1991; Graham et al., 1995) to maintain intracellular osmolality (Lang et al., 1998; Sejersted & Sjøgaard, 2000; Stutzin et al., 1999). However, the appearance of endogenous taurine (i.e. without oral supplementation) has not been reported to affect plasma volume (Cuisinier et al., 2002). Theoretically, the higher plasma taurine concentrations observed following oral administration may increase the osmotic pressure in both central and peripheral sites. This may draw additional fluid into the vasculature and potentially sustain (e.g. during periods of profuse sweating) or expand plasma volume. Mechanistically, a greater taurine concentration in the plasma, increasing the osmotic gradient and expanding plasma volume is possible considering that taurine is extruded from skeletal muscle cells into extracellular compartments to prevent cell swelling (Stutzin et al., 1999). However, it should be noted that taurine has a weaker relationship with plasma volume compared to other osmolytes (Cuisinier et al., 2002) and the effect of exogenous supplementation on plasma volume in heat stressed, exercising humans has not yet been established. Furthermore, plasma volume maintenance could augment sweating via preservation of SkBF (Nagashima et al., 1998; Nielsen et al., 1984), fluid availability and supply to the sweat gland (Fortney et al., 1981; Wong & Hollowed, 2016) or a change in osmoreceptor or baroreceptor signalling (Shibasaki et al., 2011; Mack et al., 1995). Taurine's established accumulation in the interstitial fluid (Pasantes-Morales et al., 1998) could be enhanced, secondary to oral supplementation, increasing the osmotic gradient and, thus, fluid availability for eccrine gland sweat production. Nevertheless, only one study has been conducted investigating the effects of oral taurine supplementation on sudomotor function during heat stress and, therefore, this requires replication alongside elucidation of the potential mechanisms of action.

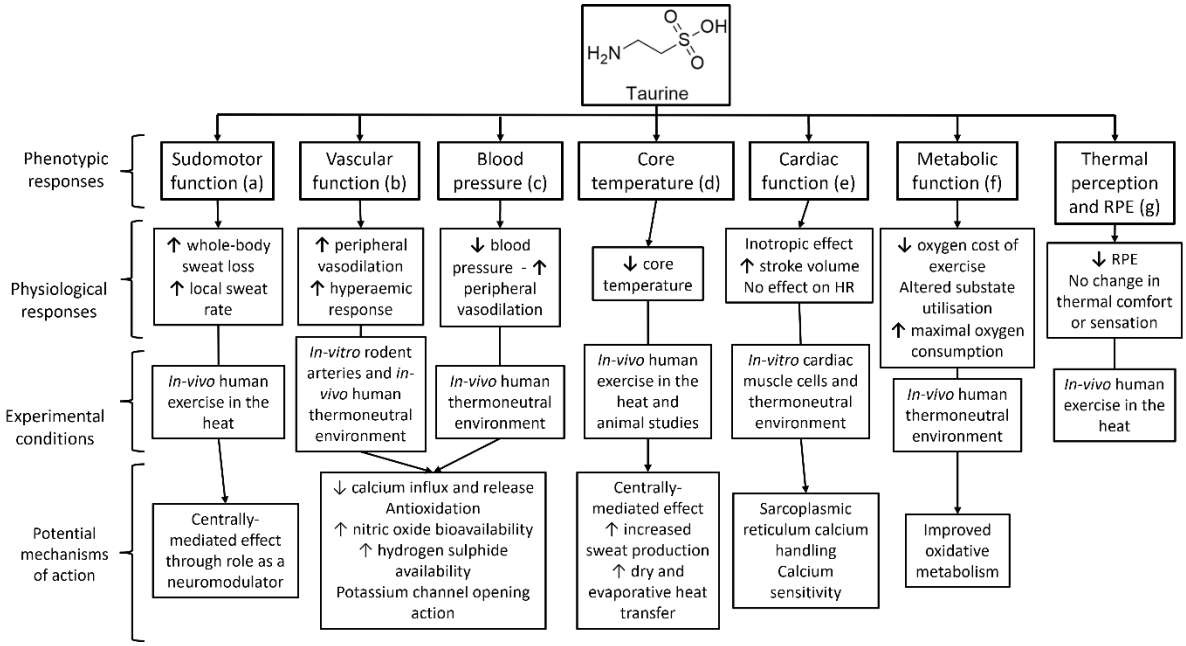


Figure 2.1. The potential effect of taurine on thermoregulatory phenotypic responses. *Letters a-g indicate where within the text each specific phenotypic response is discussed.*

2.6. Summary

Exposure to, and exercise in hot and/or humid environmental conditions often induces thermal strain, positive heat storage and rises in T_{core}. This necessitates compensatory heat loss mechanisms (i.e. thermoeffector responses), such a sweating and vasodilation to enhance dry and evaporative heat dissipation in order to maintain thermal equilibrium. These thermoeffector responses can be acutely or chronically manipulated through various interventions. The dietary supplement taurine has demonstrated the potential to augment thermal sweating and elicit vasodilation and, therefore, may be of thermoregulatory benefit in the heat. However, its vasoactive effects during heat exposure have not been characterised and only a single study has investigated its effects on sudomotor function. Consequently, it remains necessary to more comprehensively evaluate the potential thermoregulatory role of taurine supplementation in a controlled experimental setting, where sufficient control of the drivers of sweating ($\dot{H}_{prod}$ and $\dot{E}_{req}$) is permitted. Furthermore, the effect of taurine on the sweating response across more prolonged exercise periods is unknown, as is its thermoregulatory effects at rest. Additionally, taurine appears to have other physiological roles in metabolic and cardiac function which remain to be elucidated in heat stressed individuals.

3. Chapter Three – Equations

$$\dot{H}_{prod} = \dot{M} - Wk\ [W] \quad \text{(equation 1)}$$

$\dot{H}_{prod}$, metabolic heat production; $\dot{M}$, metabolic energy expenditure determined using measured $\dot{V}O_2$ and $\dot{V}CO_2$ within the final 1 min of each stage (equation 2); Wk, mechanical work

$$\dot{M} = \dot{V}O_2 \times \frac{\left(\left(\frac{RER-0.7}{0.3}\right)\times 21.13\right)+\left(\left(\frac{1.0-RER}{0.3}\right)\times 19.62\right)}{60} \times 1000\ [W] \quad \text{(equation 2)}$$

$\dot{V}O_2$, oxygen uptake; RER, respiratory exchange ratio

$$\dot{H}_{prod} = \frac{\dot{H}_{prod}}{BSA}\ [W/m^2] \quad \text{(equation 3; Cramer \& Jay, 2014)}$$

BSA, body surface area

$$BSA = 0.00718 \times (\text{body mass (kg)}^{0.425}) \times (\text{height (cm)}^{0.725})\ [m^2] \quad \text{(equation 4; Dubois \& Dubois, 1916).}$$

$$\text{Required work rate} = \frac{\text{Desired } \dot{H}_{prod}\ (W/m^2) - \text{y intercept}}{\text{Slope}}\ [W] \quad \text{(equation 5; Cramer \& Jay, 2014)}$$

$$\dot{E}_{req} = \dot{H}_{prod} - \dot{H}_{dry\ skin} - \dot{H}_{res}\ [W] \quad \text{(equation 6)}$$

$\dot{E}_{req}$, evaporation required to maintain heat balance; $H_{dry\ skin}$, dry heat exchange at the skin surface; H_{res}, respiratory heat loss

$$H_{dry\ skin} = C_{skin} + R_{skin} + K_{skin}\ [W] \quad \text{(equation 7)}$$

C_{skin}, convection; R_{skin}, radiation; K_{skin}, conduction

$$C_{skin} + R_{skin} = \frac{(T_{sk} - t_o)}{\left(R_{cl}\frac{1}{h \times fcl}\right)} \times A_D\ [W] \quad \text{(equation 8)}$$

T_{sk}, skin temperature; t_o, operative temperature; R_{cl}, dry heat transfer of clothing; h, combined convective heat transfer coefficient; f_{cl}, clothing area factor; A_D, body surface area

$$t_0 = \frac{h_r t_r + h_c t_a}{h_r + h_c} \text{ [W]} \quad \text{(equation 9)}$$

h_r, radiative heat transfer coefficient; t_r, radiant temperature; h_c, convective heat transfer coefficient; t_a, ambient air temperature

$$h = h_c + h_r \text{ [W/m}^2\text{/K]} \quad \text{(equation 10)}$$

$$h_c = 8.3 \times v_{air}^{0.6} \text{ [W/m}^2\text{/K]} \quad \text{(equation 11)}$$

V_{air}, ambient air velocity

$$h_r = 4\,\varepsilon\,\sigma\,\frac{A_r}{A_D}\left(273.2 + \frac{T_{sk} + t_r}{2}\right)^3 \text{ [W/m}^2\text{/K]} \quad \text{(equation 12)}$$

ε, non-dimensional emissivity of the body surface; σ, Stefan-Boltzmann constant; A_r/A_D, fraction of the body surface participating in radiative heat transfer

$$H_{res} = C_{res} + E_{res} \text{ [W]} \quad \text{(equation 13)}$$

C_{res}, convective respiratory heat loss; E_{res}, evaporative respiratory heat loss

$$C_{res} = 0.001516 \times \dot{M}(28.56 + 0.641 \times Pa - 0.885 \times t_a) \text{ [W]} \quad \text{(equation 14)}$$

P_a, vapor pressure of inspired air

$$Pa = \frac{6.116441 \times 10^{\left(\frac{7.5911386 \times t_a}{t_a + 240.7263}\right)} \times \frac{\%RH}{100}}{10} \text{ [kPa]} \quad \text{(equation 15)}$$

%RH, relative humidity

$$E_{res} = 0.00127 \times M(59.34 + 0.53 \times t_a - 11.63 \times Pa \text{ [W]} \quad \text{(equation 16)}$$

$$\dot{E}_{skin} = \text{delta body mass loss} \times \frac{\lambda}{1000} \text{ [kJ]} \quad \text{(equation 17)}$$

λ, latent heat of vaporisation of sweat (2426 J/g).

$$\dot{E}_{max} = \frac{(P_{skin,sat} - P_a)}{R_{e,cl} + \frac{1}{h_e \times f_{cl}}} \times A_D \ [W] \quad \text{(equation 18)}$$

$\dot{E}_{max}$, maximal evaporative heat transfer capacity; $P_{skin,sat}$, vapour pressure at the skin surface while saturated in sweat; $R_{e,cl}$, evaporative resistance of clothing; h_e, evaporative heat transfer coefficient

$$P_{skin,sat} = \frac{\frac{(EXP(18.956-(4030.18)}{T_{sk}+235}}{10} \ [kPa] \quad \text{(equation 19)}$$

$$h_e = (16.5 \times h_c) \ [W] \quad \text{(equation 20)}$$

$$S = \ time \times \frac{\dot{H}_{prod} - \dot{H}_{dry\ skin} - \dot{H}_{evap\ skin} - \dot{H}_{res}}{1000} \ [kJ] \quad \text{(equation 21)}$$

$$H_{evap\_skin} = WBSR \times \lambda \times \frac{S_{eff}}{60} \ [W] \quad \text{(equation 22)}$$

WBSR, whole-body sweat rate based on body mass changes over time (g/min); S_{eff}, sweating efficiency

$$S_{eff} = 1 - \frac{\omega_{req}{}^2}{2} \ [ND] \quad \text{(equation 23)}$$

ω_{req}, required skin wettedness

$$\omega_{req} = \frac{\dot{E}_{req}}{\dot{E}_{max}} \ [ND] \quad \text{(equation 24)}$$

$$T_{sk} = \ (T_{chest} + T_{arm}) \times 0.3 + \left(T_{thigh} + T_{calf}\right) \times 0.2 \ [°C] \quad \text{(equation 25; Ramanathan, 1964)}$$

T_{chest}, chest skin temperature; T_{arm}, arm skin temperature; T_{thigh}, thigh skin temperature; T_{calf}, calf skin temperature

$$\text{Local sweat rate} = \frac{\text{pre to post change in patch mass (mg)}}{[5\ (cm) \times 5.5\ (cm)] \times 5\ (min)} \ [mg/cm^2/min] \quad \text{(equation 26; Baker et al., 2018)}$$

$$SkBF = \frac{(\frac{1}{SH} \times \dot{H}_{prod})}{(T_{core} - T_{sk})} \ [ND] \quad \text{(equation 27; Sawka \& Young, 2006)}$$

SkBF, skin blood flow; SH, specific heat of the blood (~1 kcal/°C); T_{core}, core temperature and $\dot{H}_{prod}$ is expressed in kcal/min$\mathrm{SkBF} = \frac{(\frac{1}{\mathrm{SH}} \times \dot{\mathrm{H}}_{\mathrm{prod}})}{(\mathrm{T}_{\mathrm{core}} - \mathrm{T}_{\mathrm{sk}})}$ [ND]

$\mathrm{CVC} = \frac{\text{perfusion units}}{\mathrm{MAP}} \times 100\ [\mathrm{mmHg}]$ (equation 28; Stapleton et al., 2014b)

CVC, cutaneous vascular conductance; MAP, mean arterial pressure

$\mathrm{MAP} = \mathrm{DBP} + (\frac{1}{3} \times \text{pulse pressure})\ [\mathrm{mmHg}]$ (equation 29; Stapleton et al., 2014b)

DBP, diastolic blood pressure

$\text{Pulse pressure} = \mathrm{SBP} - \mathrm{DBP}\ [\mathrm{mmHg}]$ (equation 30; Stapleton et al., 2014b)

SBP, systolic blood pressure

Vasodilation% = ($[D_{max} - D_{baseline}]/D_{baseline}$) × 100 (equation 31; Atkinson & Batterham, 2013)

D_{max} (mm), maximum artery diameter achieved post-IHG; $D_{baseline}$ (mm), resting diameter pre-IHG

$\text{Shear rate} = (4 \times V_{\mathrm{mean}})/\mathrm{D}\ [1/\mathrm{s}]$ (equation 32; Thijssen et al., 2019)

V_{mean}, time-averaged mean velocity of the blood expressed as cm/s; D (mm), artery diameter

$\text{Blood flow} = (V_{\mathrm{mean}}) \times \pi + \left(\frac{\mathrm{D}}{2}\right) 2 \times 60\ [\mathrm{mL/min}]$ (equation 33; Dulaney et al., 2023)

π, a mathematical constant; D, diameter of the artery in cm; 60 is a constant employed to convert the units to mL/min.

See Cramer & Jay (2019) for further information regarding partitional calorimetry, equations 1-2 and 6-24, and their components. Additional references are provided next to their respective equations.

4. Chapter Four – The effect of dietary supplements on endurance exercise performance and core temperature in hot environments: a meta-analysis and meta-regression.

The study that comprises Chapter Four has been published in Sports Medicine. Chapter Four is identical to the published version, with the only adaptations due to required formatting alterations.

Reference: Peel, J. S., McNarry, M. A., Heffernan, S. M., Nevola, V. R., Kilduff, L. P., & Waldron, M. (2021). The effect of dietary supplements on endurance exercise performance and core temperature in hot environments: A meta-analysis and meta-regression. *Sports Medicine*, *51*(11), 2351-2371.

4.1. Abstract

Background The ergogenic effect of dietary supplements on endurance exercise performance are well-established; however, their efficacy in hot environmental conditions has not been systematically evaluated.

Objectives The objectives of the present review were two-fold: 1) To meta-analyse studies investigating the effects of selected dietary supplements on endurance performance and T_{core} responses in the heat. Supplements were included if they were deemed to: a) have a strong evidence base for 'directly' improving thermoneutral endurance performance, based on current position statements, or b) a proposed mechanism of action that related to modifiable factors associated with thermal balance. 2) To conduct meta-regressions to evaluate the moderating effect of selected variables on endurance performance and T_{core} responses in the heat following dietary supplementation.

Methods A search was performed using various databases in May 2020. After screening, 25 peer-reviewed articles were identified for inclusion, across three separate meta-analyses: 1) exercise performance; 2) end T_{core}; and 3) submaximal T_{core}. The moderating effect of several variables were assessed via sub-analysis and meta-regression.

Results Overall, dietary supplementation had a *trivial* significant positive effect on exercise performance (Hedge's g = 0.18, 95% CI 0.007-0.352, p = 0.042), a *trivial* non-significant

positive effect on submaximal T_{core} (Hedges' g = 0.18, 95% CI -0.021-0.379, p = 0.080) and a *small* non-significant positive effect on end T_{core} (Hedges' g = 0.20, 95% CI -0.041-0.439, p = 0.104) in the heat. There was a non-significant effect of individual supplements on exercise performance (p = 0.973) and submaximal T_{core} (p = 0.599). However, end T_{core} was significantly affected by supplement type (p = 0.003), which was attributable to caffeine's *large* significant positive effect (n = 8; Hedge's g = 0.82, 95% CI 0.433-1.202, p < 0.001) and taurine's *medium* significant negative effect (n = 1; Hedges' g = -0.96, 95% CI -1.855- -0.069, p = 0.035).

Conclusion Supplements, such as caffeine and nitrates, do not enhance endurance performance in the heat, with caffeine also increasing T_{core} responses. Some amino acids might offer the greatest performance benefits in the heat. Exercising in the heat negatively affected the efficacy of many dietary supplements, indicating that further research is needed and current guidelines for performance in hot environments likely require revision.

4.2. Introduction

The ergogenic effects of a number of dietary supplements on endurance exercise performance are well-established (Christensen et al., 2017; Doherty & Smith, 2004; McMahon et al., 2017b; Schubert & Astorino, 2013; Southward et al., 2018). Indeed, recent position statements by the International Olympic Committee (IOC; Maughan et al., 2018), American College of Sports Medicine (ACSM; Thomas et al., 2016) and the Union of European Football Associations (UEFA; Collins et al., 2020) provide specific recommendations for certain performance enhancing dietary supplements that are thought to have sufficient evidence for use by endurance athletes during training and competition. In tactical occupational settings, official policy information on the use of dietary supplements is often provided (United States Army Field Manual 7-22) however, specific guidance on ergogenic aids is not. Despite this, the use of supplements among military personnel in training (Austin et al., 2016; Casey et al., 2014) and during operations (Boos et al., 2011; Boos et al., 2010) has been well reported. Whilst it has been recognised that contextual factors should be considered when selecting dietary supplements (United States Army Field Manual 7-22; Maughan et al., 2018), there is limited guidance on this relating to endurance exercise performed in hot environments. This is particularly surprising, given that many endurance events and major international competitions take place in a combination of hot and humid conditions (Ely et al., 2008; Racinais et al., 2015). For example, the forthcoming Tokyo 2021 Olympic Games are expected to take place in air temperatures exceeding 30 °C, with a humidity index of ~ 38 (Gerrett et al., 2019; Kashimura et al., 2016). Furthermore, military training and operations are also often conducted in extreme

environments, in combination with prolonged endurance activity (Parsons et al., 2019; World & Booth, 2008).

Physical capacity is markedly impaired with increasing ambient temperature and humidity (Galloway & Maughan, 1997; Hargreaves, 2008; Junge et al., 2016; Maughan et al., 2012), leading to thermoregulatory strain and early onset fatigue, for a variety of physiological reasons (Cheuvront et al., 2010; Galloway & Maughan, 1997; González-Alonso et al., 1999; González-Alonso et al., 2008; Hargreaves & Febbraio, 1998; Nybo, 2010; Nybo et al., 2011; Périard et al., 2011; Thompson, 2006; Tucker et al., 2004). To perform optimally, environmental conditions - and their interaction with dietary supplement choices - must be carefully considered. Improper preparation for exercise in the heat can not only have detrimental effects on performance but can also lead to severe heat illness, and even death, in some extreme cases (Armed, 2017; Bricknell, 1996; Cox et al., 2016; Howe & Boden, 2007). Therefore, a more comprehensive understanding of the effects of commonly used dietary supplements on physical performance and thermoregulation during exercise in the heat is necessary and could lead to safer and/or more efficacious heat preparation strategies.

The major limiting factors during exercise in the heat are linked to inexorable increases in T_{core} (González-Alonso et al., 1999), cardiovascular strain (Cheuvront et al., 2010) and/or reductions in central drive (Nybo, 2010). Conceptually, regarding most endurance athletes and military personnel, the capacity to dissipate heat and offset one, or all, of these eventualities in hot environments predominantly occurs via three modifiable factors: lowered metabolic heat production, enhanced skin vasodilation (i.e. convective heat loss) or sweating (i.e. evaporative heat transfer; Benzinger et al., 1961a; Gagge & Gonzales, 1996; Wendt et al., 2007). The two supplements deemed to have the strongest empirical evidence to support these mechanisms (Maughan et al., 2018), and reportedly served to aid endurance exercise performance in temperate conditions, are caffeine (1,3,7-trimethylxanthine; Doherty & Smith, 2004; Ganio et al., 2009) and dietary nitrate (NO_3^-; McMahon et al., 2017b). Mechanistically, there is a sound theoretical basis for both caffeine and nitrate supplementation to offset fatigue in the heat through increased central drive (caffeine; Davis et al., 2003), and NO's action on eccrine sweat gland function and subcutaneous microvascular control (nitrate; Fujii et al., 2014; McGinn et al., 2014; Welch et al., 2009). However, numerous studies have reported negative or null performance and thermoregulatory effects for both of these supplements during exercise in the heat (Cheuvront et al., 2009; Fowler et al., 2020; Hanson et al., 2019; McQuillan et al., 2018; Roelands et al., 2011).

The apparent failure of these well-evidenced supplements to produce an ergogenic effect in the heat is largely unexplained but could be due to the differing physiological demands of

exercise in the heat, and a combination of factors limiting exercise tolerance in a hot environment (Cheuvront et al., 2010; González-Alonso et al., 2008; Nybo, 2010; Nybo et al., 2011; Thompson, 2006; Tucker et al., 2004). It is also possible that ancillary physiological effects (i.e. on T_{core} and blood pressure) of selected supplements have not been fully considered in accordance with environmental constraints and could inadvertently exacerbate symptoms of heat stress, which has been inferred from laboratory-based studies of caffeine (Hanson et al., 2019) and nitrate supplementation (Amano et al., 2018). A similar line of reasoning can be applied to most other dietary supplements, based on the poor knowledge of their specific effects on thermoregulatory processes and subsequent ergogenic effects in the heat. Indeed, a number of alternative supplements have received some attention for their use in hot environments. For instance, supplementation with branched-chain amino acids (BCAAs; Mittleman et al., 1998), tyrosine (Tumilty et al., 2011) and taurine (Page et al., 2019) have been shown to extend time-to-exhaustion (TTE) in the heat, indicating that amino acids have ergogenic potential in hot conditions, yet these are not among those most commonly selected for training or competition purposes (Knapik et al., 2016; Wardenaar et al., 2017). Irrespective of the exact reasons for the apparent inconsistent findings within the published literature, there has not yet been a systematic evaluation of dietary supplements for endurance athletes and/or military personnel in the heat, which is necessary to clarify the most ergogenic options and least likely to contribute to rises in T_{core}.

Therefore, the aims of this meta-analysis were to investigate the effects of selected dietary supplements on endurance performance in the heat, as well as the associated T_{core} responses. The ergogenic effect of macronutrients (Burke, 2001; Carter et al., 2003; Cathcart et al., 2011) and eu/hyper-hydration (Burke, 2001; Casa et al., 2010; Hoffman et al., 2018; Maughan & Shirreffs, 2004; Morris et al., 2015; Sawka et al., 2001; Tan & Lee, 2015) on endurance exercise performance in the heat have been well-established and do not require revisiting here. However, the control of these factors among studies evaluating the efficacy of dietary supplements can be inconsistent, often precluding direct comparisons. Likewise, the training and acclimation status of participants has a significant effect on their thermoregulatory control and subsequent heat tolerance (Ravanelli et al., 2018), as does the selected mode of exercise (i.e. time-trial [TT] *vs* TTE; Schlader et al., 2011). This will affect behaviour and pacing during performance (Racinais et al., 2015), yet these details appear to lack appropriate attention and have been largely overlooked in current consensus guidelines (Maughan et al., 2018; Thomas et al., 2016). Therefore, to understand the potential effects of dietary supplements on endurance performance in the heat, these factors were considered as potential moderating variables, forming part of the current meta-regression analysis.

4.3. Methods

4.3.1. Search strategy

All literature that investigated the effects of dietary supplementation on exercise performance in a hot environment was searched and obtained using the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines, with a predetermined search strategy (Moher et al., 2015). Medical subject headings (MeSH) terms were left active during the searches. There was no limit on the status, date or language of the publication. The single paper published in a language other than English was translated digitally using two separate translation software programs; Google Translate and DeepL Translator (DeepL GmbH, Cologne, Germany). The final Boolean searches were performed in PubMed and SPORTDiscus (EBSCO) on 6th May 2020. The search terms used were '(dietary supplements OR dietary supplementation OR nutritional supplements OR nutritional supplementation OR supplements OR supplementation OR ergogenic OR ergogenic aids OR caffeine OR creatine OR nitrate OR sodium bicarbonate OR beta-alanine) AND (heat OR cold OR temperature OR body temperature regulation)' and all combinations were searched independently. The dietary supplements; caffeine, creatine, nitrate, sodium bicarbonate and beta-alanine were searched individually as they have been recognised by the IOC (Maughan et al., 2018) and ACSM position statements (Thomas et al., 2016) as having the greatest empirical evidence for their ergogenic effects in a thermoneutral environment and are, therefore, relevant to review in the heat. As there is no *a-priori* list of dietary supplements that are ergogenic through their effect on thermal balance, no other supplements were searched individually by name. All relevant supplements should be returned under the other search terms. Two investigators (JP and MW) verified the search terms and the accuracy of the returned results.

4.3.2. Study selection

Following the identification of all articles, the titles and abstracts were screened for inclusion by two reviewers and any duplicates removed. 'Other sources' were also identified, such as through social media (Twitter or 'X'). The reference lists of the initial papers were reviewed independently by two investigators (JP and MW). The remaining articles were then assessed separately (and without influence) by JP and MW against the inclusion and exclusion criteria. There was 100% agreement in study selection between the two reviewers. Papers were required to have been published in a peer-reviewed journal as original research articles with a cross-over, randomised control trial or an independent groups design. They must also have included a control or placebo group and participants were required to be healthy adults (≥ 18 years). To be included in this analysis, the studies must have passed through one of two filter

points: (1) administered a dietary supplement recognised by the IOC (Maughan et al., 2018) and ACSM position statements (Thomas et al., 2016) as having a strong evidence base for 'direct' improvements to performance; or (2) have a proposed ergogenic mechanism of action either directly or indirectly related to modifiable factors associated with thermal balance (i.e. SkBF, sweating, exercise efficiency). The studies must also have: (1) administered a dietary supplement (by the definition below); (2) evaluated endurance exercise protocols performed for > 75 s; and (3) been conducted in an ambient dry-bulb temperature of ≥ 30 °C in either a laboratory or field setting. Of the remaining papers, a number were further removed for the reasons outlined in Figure 4.1. This largely comprised papers that included supplements that were: co-ingested; a drug; not orally administered; or a macro-nutrient (or had a mechanism of action which was considered to be directly related to hydration or gut function). Other reasons were the absence of a performance measure or one not adhering to the above definition; or environmental issues.

A dietary supplement was defined by adapting the IOC position statement (Maughan et al., 2018): a non-food, non-pharmacological, food component, nutrient or non-food compound that is purposefully orally ingested in addition to the habitual diet with the aim to 'directly' improve sports/exercise performance. The supplement is not being consumed for its indirect health benefits, its calorific value, its effects on hydration or gut function (the ergogenic mechanism of action is not through greater fluid absorption in the gut or increased gut permeability). The supplement is also legal as per the Misuse of Drugs Act 1971 (U.K. Government, 1971) and is not on the World Anti-Doping Association's prohibited substances list (WADA, 2020). 'Direct' supplements refer to those acutely enhancing performance but not solely via "*effective training, better recovery from training sessions, optimising mass and body composition, or reducing risks of injury and illness*".

Endurance performance encompasses a variety of activities, and the current analysis allowed for three forms of exhaustive exercise of any mode; TTE, TT and power output during closed loop tasks (i.e. Intermittent Sprint Tests [IST]). Overall effects (i.e. combinations of all modalities) were considered for the analysis. Any forms of exercise that were either not exhaustive or performed for < 75 s were removed. This was based on the knowledge that exercise performed for ≥ 75 s has predominant contributions from aerobic metabolism, even at maximal intensities and irrespective of ambient temperature (Baker et al., 2010; Gastin, 2001; Waldron et al., 2015).

4.3.3. Data extraction and quality assessment

Data were extracted and entered into a custom-designed Microsoft Excel spreadsheet. Extracted data included: (1) characteristics of the sample (sex, age, health, training and heat acclimation/acclimatisation status); (2) study design; (3) supplement, dose and timing of intake; (4) food and fluid intake before and during exercise i.e. hydration status, food intake before exercise and fluid ingestion during exercise; (5) environmental conditions (temperature and humidity); (6) performance outcomes; (7) end and submaximal T_{core} (rectal, gastrointestinal, oesophageal or tympanic); and (8) bias. Risk of bias was assessed independently by two investigators (JP and MW) according to Cochrane collaboration guidelines (Higgins et al., 2019). Where details of the study were unclear, the authors of the relevant papers were consulted for specific information or to clarify the method that was used. There was 100% agreement between the authors concerning the outcome of this quality assurance procedure, hence, it was not considered necessary to include a third independent reviewer. Standardised mean difference (SMD) was used to compare the results between studies utilising different protocols and measures. There were three outcome measures for this meta-analysis: (1) exercise performance; (2) T_{core} reported at the end of the exercise protocol, hereafter referred to as 'end T_{core}'; and (3) T_{core} reported at the mid-point of the exercise protocol, hereafter referred to as 'submaximal T_{core}'.

4.3.4. Statistical analysis

Data analysis was performed by one author (JP). Data were extracted from the qualifying papers in the form of a mean, standard deviation (SD) and sample size (*n*) for the meta-analysis. Publicly available software (WebPlotDigitizer, Version 4.3; Rohatgi, 2017) was used to extrapolate any unreported values from the figures to mean and SD data. Authors of the original research articles were contacted for any missing data; however, were this not accessible, they were imputed using the sample pooled SD from similar included studies (Furukawa et al., 2006). Pre-to-post change scores were not used for any analysis, owing to their inconsistent availability. However, both sub-maximal and maximal T_{core} measures were reported to evaluate potential differences across stages of the exercise trials.

Three meta-analyses were conducted, one for each outcome measure. These were performed in RStudio (Harrer et al., 2019; Team, 2013) and included 25, 24 and 20 comparison groups, for the exercise performance, end T_{core} and submaximal T_{core} meta-analyses, respectively. Not all studies reported end and submaximal T_{core}, hence, they were excluded from the analysis. All data were analysed with a random-effects model, with heterogeneity assessed using the I^2 statistic. Outliers were detected using a function in RStudio and influence on analysis

investigated. Publication bias was accounted for by funnel plots and conducting Egger's test and subsequently Duval and Tweedie's Trim and Fill procedure, when indicated (Rothstein et al., 2005). Hedges' *g* and 95% Confidence Intervals (CI) were used to express SMD between dietary supplementation and placebo groups across studies. Sub-analysis of the different supplements included, and of the different exercise modalities utilised, were conducted for all three meta-analyses. Meta-regressions were also conducted to determine the effect of candidate moderators on exercise performance and T_{core} outcomes, as reported in each study: training status (highly trained *vs* recreationally active); heat acclimation status (heat acclimated *vs* non-heat acclimated); hydration status (euhydrated *vs* hypohydrated); fluid ingestion during exercise (fluid ingestion *vs* no fluid ingestion); fasted *vs* fed state; exercise beforehand (exercise *vs* no exercise); heat exposure beforehand (heat exposure *vs* no heat exposure); duration of performance protocol; and total exercise duration. The thresholds for the magnitude of effects were < 0.2, 0.2, 0.5 and 0.8 for *trivial, small*, *medium* and *large* effects, respectively (Rosenthal & Rosnow, 2008). Alpha (α) was set at $P \leq 0.05$ for all analyses.

4.4. Results

4.4.1. Study selection

The initial searches retrieved 25,453 articles, plus one additional study through social media (Twitter or 'X'). These were reduced to 7,534 after removal of duplicates. After further screening and removal of reviews, animal studies and other irrelevant papers, 91 articles remained. Searches of the reference lists within those 91 reported studies provided five further papers. Of the 96 articles, 61 were removed based on the inclusion criteria and a further 10 were removed due to having: duplicate data with another paper, no full-text or no extractable data. This left 25 papers, of which 25, 24 and 20 papers were included in the exercise performance, end T_{core} and submaximal T_{core} analyses, respectively (Figure 4.1).

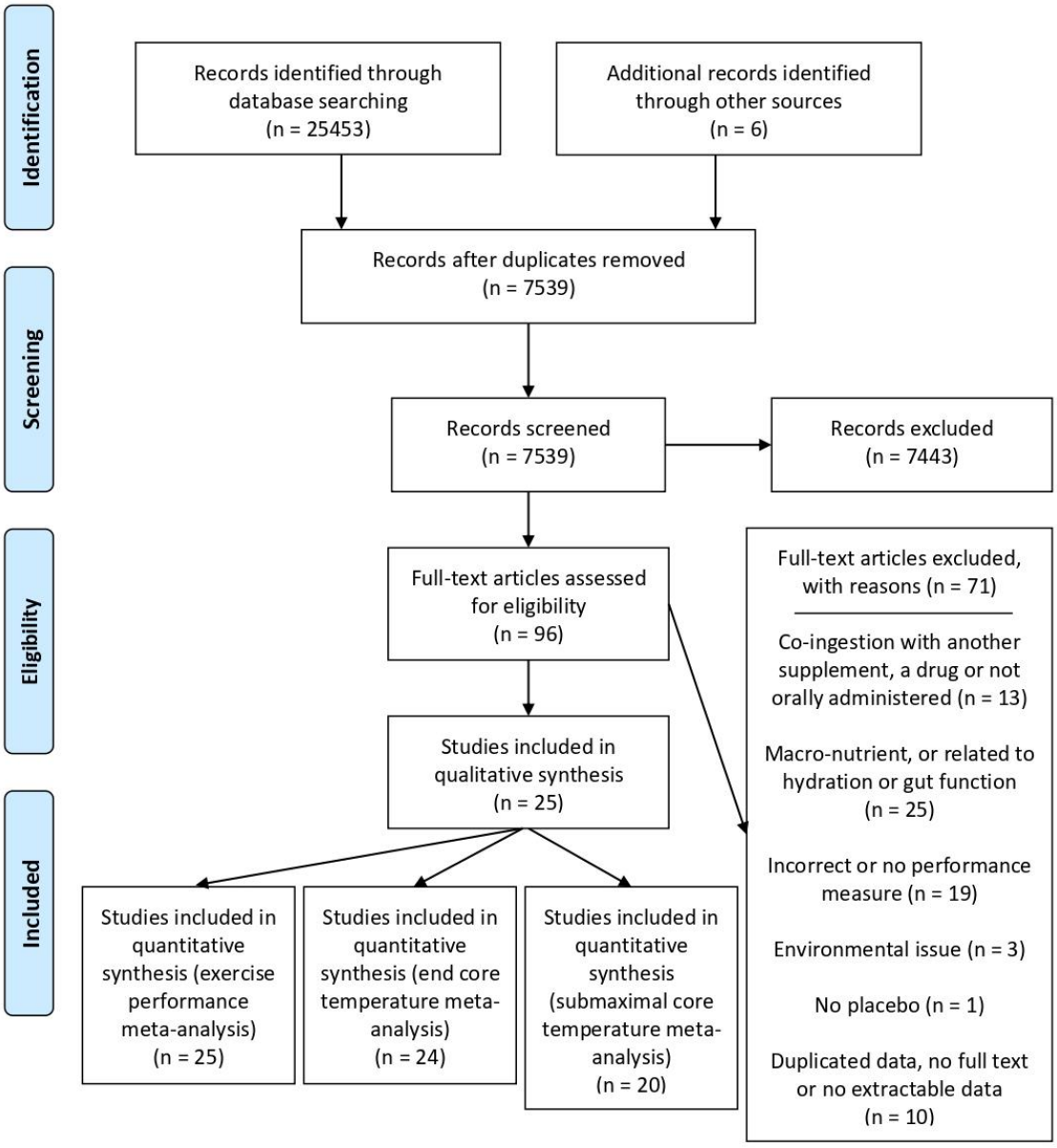


Figure 4.1. The process of study selection.

4.4.2. Study characteristics

The characteristics of the 25 included studies have been summarised in Table 4.1. The studies included a total of 272 participants, comprising both males and females (males 88%; both males and females 12%) of varying training (highly trained 56%; recreationally active 44%) and heat acclimation statuses (heat acclimated 16%; non-heated acclimated 56%; unreported 28%). Twenty-four of the studies were cross-over designs, whilst one study was an independent groups' design (Table 4.1). Nine different types of supplements were included (caffeine, creatine, nitrate, BCAAs, tyrosine, vitamin E, Eurycoma longifolia Jack, taurine and

polyphenols) in varying doses. These were a combination of single acute doses (*n* = 18; 72%) and chronic administration (*n* = 7; 28%). The performance measures included were TT (52%), TTE (44%) and IST (4%). The measures of T_{core} were rectal (64%), tympanic (12%), oesophageal (4%), gastrointestinal (16%) and unreported (4%). Ambient temperature (mean 33.2°C; range 30 to 42°C), RH% (mean 47%; range 20 to 70%) and exercise time (mean 50 min; range 2 to145 min) are reported herein. There were no adverse health-related events noted in any of the studies.

Table 4.1. Summary of studies included in the meta-analyses (n = 25).

Study	Design	Sample	Supplement, dose and timing	Temperature and relative humidity	Core temperature method	Exercise performance type	Outcome
Beaumont and James (2017)	Double-blind, randomised, repeated measures, cross-over	Healthy, recreationally active, non-heat acclimated males (*n* = 8). Age 22 ± 1 years	Caffeine 6 mg·kg^{-1} (60 min pre-exercise)	30 °C 50% RH	Gastrointestinal every 5 min (ECT + SCT)	60 min cycling @ 55% W_{max} followed by 30 min TT	NS ˜3% ↑ in TT performance
Cheuvront et al. (2009)	Double-blind, randomised, cross-over	Healthy, physically active, moderately fit, non-heat acclimated males (*n* = 10). Age 23 (18-37) years	Caffeine 9 mg·kg^{-1} (timing not mentioned)	40 °C 20-30% RH	Rectal every 5 min (ECT + SCT)	30 min cycling @ 50% $\dot{V}O_{2peak}$ followed by 15 min TT	NS ˜2.4% ↑ in TT performance
Ferreira et al. (2005)[b]	Double-blind, randomised, cross-over	Well-trained, heat acclimated, male cyclists (*n* = 8). Age 23.9 ± 8.6 years	Caffeine 5 mg·kg^{-1} (60 min pre-exercise)	30 °C average, ranged from 28.5-32 °C 71-78% RH	Tympanic pre and post exercise (ECT)	45 km cycling TT	NS ˜4.2% ↑ in TT performance
Ganio et al. (2011)	Double-blind, randomised, cross-over	Healthy, trained, non-heat acclimated male cyclists (*n* = 11). Age 25 ± 6 years	Caffeine 3 mg·kg^{-1} (60 min pre-exercise)	33 °C 41% RH	Rectal every 15 min (ECT + SCT)	90 min cycling @ 65% thermoneutral $\dot{V}O_{2max}$ followed by 15 min TT	NS ˜6.3% ↑ in TT performance
Hanson et al. (2019)	Single-blind, randomised, cross-over	Trained male (*n* = 6) and female (*n* = 4) endurance runners (n = 10). Age 26 ± 9 years	Caffeine 6 mg·kg^{-1} (60 min pre-exercise)	30.6 °C 50% RH	Gastrointestinal every 1 km (ECT + SCT)	10 km running TT	NS ˜0.9% ↑ in TT performance

Ping et al. (2010)[b]	Double-blind, randomised, cross-over	Recreational, heat acclimated male runners (*n* = 9). Age 25.4 ± 6.9 years	Caffeine 5 mg·kg^{-1} (60 min pre-exercise)	31 °C 70% RH	Rectal every 10 min (ECT)	Treadmill running @ 70% $\dot{V}O_{2max}$	Significant ˜27.4% ↑ in TTE
Pitchford et al. (2014)	Double-blind, randomised, counterbalanced, cross-over	Highly-trained, non-heat acclimated male cyclists (*n* = 9). Age range 22-42 years	Caffeine 3 mg·kg^{-1} (90 min pre-exercise)	35 °C 25% RH	Gastrointestinal continuously (ECT + SCT)	Total work cycling TT	NS ˜6.9% ↑ in TT performance
Roelands et al. (2011)	Double-blind, randomised, cross-over	Healthy, trained, non-heat acclimated males (*n* = 8). Age 23 ± 5 years	Caffeine 6 mg·kg^{-1} (60 min pre-exercise)	30 °C 50-60% RH	Rectal every 5 min (ECT + SCT)	60 min cycling @ 55% W_{max} followed by total work TT	NS ˜3% ↓ in TT performance
Suvi et al. (2017)[a b]	Double-blind, randomised, cross-over	Healthy, physically active, non-heat acclimated males (*n* = 13) and females (*n* = 10; *n* = 23). Age 24.9 ± 4.1 *vs* 22.5 ± 2 years	Caffeine 6 mg·kg^{-1} (4 mg·kg^{-1} 60 min and 2 mg·kg^{-1} 0 min pre-exercise)	42 °C 20% RH	Measured but no extractable data	50 min treadmill walking @ 60% thermoneutral $\dot{V}O_{2peak}$ followed by TTE	NS ˜4.3% ↓ in TTE
Kilduff et al. (2004)	Double-blind, randomised, independent design	Endurance-trained, non-heat acclimated males (*n* = 11 *vs* 10; *n* = 21). Age 27 ± 5 *vs* 27 ± 4 years	Creatine 159.6 g (7 x 22.8 g·d^{-1})	30.3 °C 70% RH	Rectal every 5 min (ECT + SCT)	Cycling @ incremental work rate at 60-90 rpm	NS ˜3% ↓ in TTE
Fowler et al. (2020)	Double-blind, randomised, cross-over	Healthy, physically inactive, non-heat acclimated males (*n* = 11). Age 25 ± 5 years	Nitrate (NO_3^-) 46 mmol (5 x 9.2 mmol·d^{-1})	35 °C 28% RH	Rectal every 1 min (ECT + SCT)	Cycling @ thermoneutral gas exchange threshold at 70 rpm	NS ˜9.7% ↑ in TTE
Kent et al. (2018)	Double-blind, repeated measures,	Endurance-trained male cyclists (*n* =	Nitrate (NO_3^-) 26 mmol (2 x 6.5 mmol·d^{-1} and 13	35 °C 48% RH	Gastrointestinal every 20% work	Total work cycling TT	NS ˜3.1% ↑ in TT performance

	counter-balanced, cross-over	12). Age 26.6 ± 4.4 years	mmol 120 min pre-exercise)		rate (ECT + SCT)		
McQuillan et al. (2018)	Double-blind, randomised, cross-over	Healthy, well-trained endurance male cyclists (*n* = 8). Age 25 ± 8 years	Nitrate (NO_3^-) 24 mmol (2 x 8 mmol·d^{-1} and 8 mmol 90 min pre-exercise)	35 °C 60% RH	Rectal continuously (ECT + SCT)	20 min cycling @ 40-60% PPO followed by 4 km TT	NS ˜0.3% ↑ in TT performance
Smith et al. (2019)[b]	Double-blind, randomised, counterbalanced, cross-over	Recreationally-trained males (*n* = 12), Age 22 ± 4 years	Nitrate (NO_3^-) 6.2 mmol (180 min pre-exercise)	30 °C 70% RH	Tympanic post IST (ECT)	20 x 6s sprints (114s active recovery)	NS ˜1.5% ↓ in mean power output
Cheuvront et al. (2004)	Cross-over	Healthy, physically active, moderately fit, heat acclimated males (*n* = 7). Age 21 ± 2 years	BCAAs 14 g·kg^{-1} (0 min pre- and during exercise)	40 °C 20% RH	Rectal every 10 min (ECT + SCT)	60 min cycling @ 50% $\dot{V}O_{2peak}$ followed by 30 min TT	NS ˜14.3% ↑ in TT performance
Mittleman et al. (1998)	Double-blind, cross-over	Healthy, moderately-trained males (*n* = 7) and females (*n* = 6; *n* = 13). Age 24 ± 2.9 *vs* 25.6 ± 7 years	BCAAs Females (9.4 g) and males (15.8 g; 5 mL·kg^{-1} of 5.88 g·L^{-1} (Every 60 min at rest and 30 min during exercise)	34.4 °C 39% RH	Oesophageal every 5 min (ECT + SCT)	Cycling @ 40% $\dot{V}O_{2peak}$	Significant ˜11.1% ↑ in TTE
Watson et al. (2004)	Double-blind, randomised, cross-over	Healthy, endurance exercising, non-heat acclimated males (*n* = 8). Age 28.5 ± 8.2 years	BCAAs 4 x 250 ml at 12 g·L^{-1} (30 min intervals pre-exercise and 150 ml every 15 min during exercise)	30 °C 38% RH	Rectal every 10 min (ECT + SCT)	Cycling @ 50% $\dot{V}O_{2peak}$	NS ˜6.6% ↑ in TTE

Coull et al. (2016)	Double-blind, counter-balanced, cross-over	Recreationally active, non-heat acclimated males (*n* = 8). Age 23 ± 1 years	Tyrosine 150 mg·kg^{-1} (60 min pre-exercise)	40 °C 30% RH	Rectal every 5 min (ECT + SCT)	60 min treadmill walk followed by 2.4 km TT wearing a 25 kg backpack	NS ˜5% ↑ in TT performance
Tumilty et al. (2011)	Double-blind, randomised, cross-over	Healthy, endurance exercising, non-heat acclimated males (*n* = 8). Age 32 ± 11 years	Tyrosine 150 mg·kg^{-1} (60 pre-exercise)	30 °C 60% RH	Rectal every 10 min (ECT + SCT)	Cycling @ 68% $\dot{V}O_{2peak}$	Significant ˜14.8% ↑ in TTE
Tumilty et al. (2014)	Double-blind, randomised, cross-over	Endurance exercising, non-heat acclimated males (*n* = 7). Age 20 (range 26) years	Tyrosine 150 mg·kg^{-1} (60 pre-exercise)	30 °C 60% RH	Rectal every 5 min (ECT + SCT)	60 min cycling @ 57% $\dot{V}O_{2peak}$ followed by total work TT	NS ˜1.1% ↑ in TT performance
Watson et al. (2012)	Randomised, counter-balanced, cross-over	Physically active, trained, non-heat acclimated males (*n* = 8). Age 23 ± 3 years	Tyrosine 150 mg·kg^{-1} (120 min, 60 min, and during)	30 °C 50% RH	Rectal every 5 min (ECT + SCT)	Cycling @ 70% $\dot{V}O_{2peak}$	NS ˜2% ↓ in TTE
Keong et al. (2006)	Double-blind, randomised, cross-over	Recreational, heat acclimated male athletes (*n* = 18). Age 24.9 ± 1.4 years	Vitamin E No dose stated (6 weeks pre-exercise)	31 °C 70% RH	Rectal every 10 min (ECT + SCT)	Treadmill running @ 70% $\dot{V}O_{2max}$	NS ˜5.3% ↑ in TTE
Muhamad et al. (2010)[b]	Double-blind, randomised, cross-over	Healthy, male recreational athletes (*n* = 12). Age 23.3 ± 3.7 years	E. longifolia Jack 1200 mg (7 x 150 mg·d^{-1} and 150 mg 60 min pre-exercise)	31 °C 70% RH	Tympanic every 10 min (ECT)	60 min treadmill running @ 60% $\dot{V}O_{2max}$ followed by 20 min TT	NS ˜3.6% ↑ in TT performance
Page et al. (2019)	Double-blind, randomised, cross-over	Healthy, non-heat acclimated males (*n*	Taurine 50 mg·kg^{-1} (120 min pre-exercise)	35 °C 40% RH	Rectal every 1 min (ECT + SCT)	Cycling @ thermoneutral ventilatory	Significant ˜11.5% ↑ in TTE

		= 11). Age 23 ± 2 years				threshold at 80 rpm	
Trinity et al. (2014)	Double-blind, randomised, cross-over	Healthy, well-trained male cyclists (*n* = 12). Age 26.8 ± 5 years	Polyphenols 25,200 ppm (7 x 3600-ppm·d^{-1})	31.5 °C 55% RH	Rectal continuously (ECT + SCT)	10 min cycling @ 60-70% $\dot{V}O_{2max}$ followed by cycling @ 100% $\dot{V}O_{2max}$	NS ˜3.5% ↓ in TTE

TT time-trial, *TTE* time-to-exhaustion, *IST* intermittent-sprint-test, *NS* non-significant, *PPO* peak power output, *ECT* end core temperature, *SCT* submaximal core temperature, *RH* relative humidity, $\dot{V}O_{2max}$ maximal oxygen uptake, $\dot{V}O_{2peak}$ peak oxygen uptake, *BCAAs* branched-chain amino acids, [b] not included in submaximal core temperature analysis, [a] not included in end core temperature analysis. The table is a reflection of participant characteristics, as reported by the authors of the original articles.

4.4.3. Meta-analysis

The results of the performance meta-analysis (*n* = 25) are reported in Figure 4.2. Overall, there was a *trivial* significant positive effect of all supplements on exercise performance compared to placebo (Hedges' *g* = 0.18, 95% CI 0.007-0.352, *p* = 0.042). The I^2 statistic demonstrated 0% heterogeneity. The results of the end T_{core} (*n* = 24) and submaximal T_{core} (*n* = 20) meta-analyses have been reported in Figure 4.3. Overall, end T_{core} had a *small* non-significant increase (Hedges' *g* = 0.20, 95% CI -0.041-0.439, *p* = 0.104), and submaximal T_{core} had a *trivial* non-significant increase (Hedges' *g* = 0.18, 95% CI -0.021-0.379, *p* = 0.080), with dietary supplementation compared to placebo, with 32.9% and 0% heterogeneity (I^2), respectively.

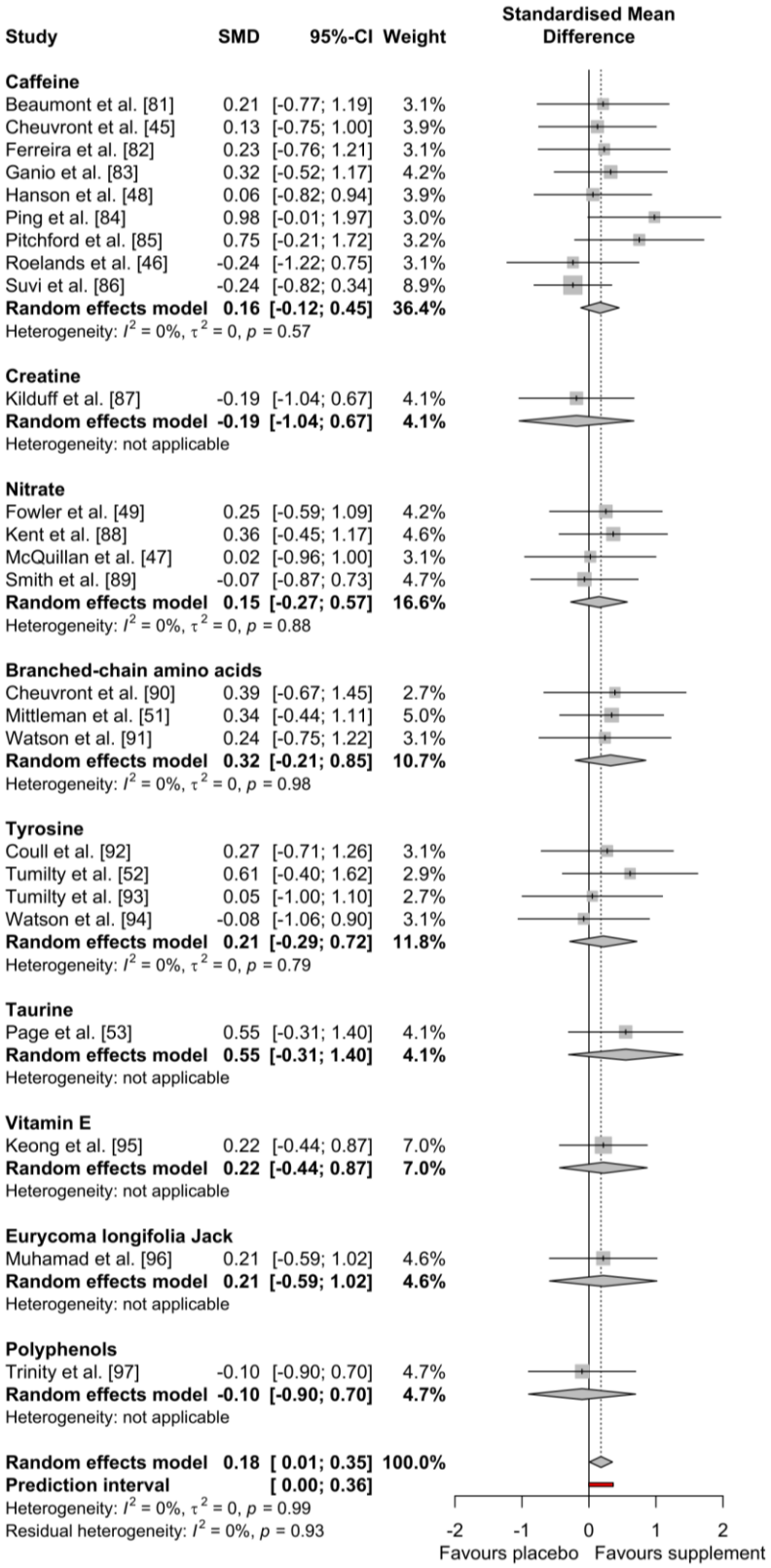


Figure 4.2. Effect of dietary supplementation on exercise performance.

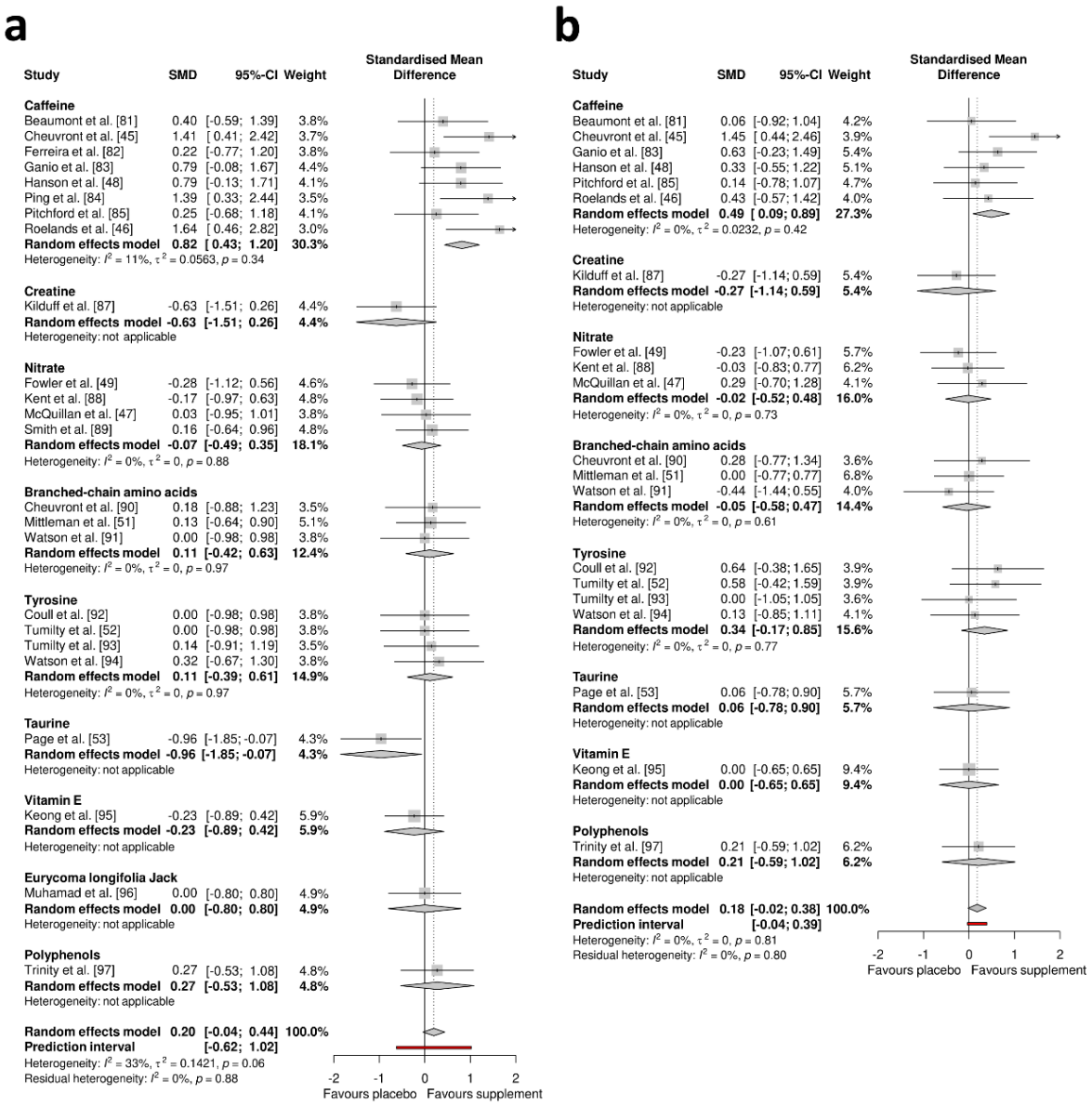


Figure 4.3. Effect of dietary supplementation on (a) end core temperature and (b) submaximal core temperature.

4.4.4. Sub-group analysis

Sub-group analyses demonstrated a non-significant effect of the different supplement categories on exercise performance (p = 0.973). Caffeine (Hedges' g = 0.16, 95% CI -0.123-0.451, p = 0.263), creatine (Hedges' g = -0.19, 95% CI -1.045-0.673, p = 0.671), nitrate (Hedges' g = 0.15, 95% CI -0.275-0.574, p = 0.490) and polyphenols (Hedges' g = -0.10, 95% CI -0.903-0.698, p = 0.802) had a *trivial* non-significant effect. Tyrosine (Hedges' g = 0.21, 95% CI -0.288-0.717, p =0.404), BCAAs (Hedges' g = 0.32, 95% CI -0.206-0.851, p = 0.232), Eurycoma longifolia Jack (Hedges' g = 0.21, 95% CI -0.590-1.016, p = 0.603) and vitamin E

(Hedges' *g* = 0.22, 95% CI -0.440-0.871, *p* = 0.520) had a *small* non-significant positive effect and taurine (Hedges' *g* = 0.55, 95% CI -0.306-1.403, *p* = 0.209) had a *medium* non-significant positive effect. Sub-group analysis of exercise modality (TTE, TT or IST) also demonstrated a non-significant effect of supplementation on exercise performance (*p* = 0.796). As shown in Figure 4.4, the use of any supplement had a *trivial* non-significant effect on TTE (Hedges' *g* = 0.17, 95% CI -0.077-0.412, *p* = 0.178) and IST performance (Hedges' *g* = -0.07, 95% CI -0.867-0.734, *p* = 0.870) and a *small* non-significant effect on TT performance (Hedges' *g* = 0.22, 95% CI -0.040-0.475, *p* = 0.097).

Sub-group analysis demonstrated a significant effect of the different supplement categories on end T_{core} (*p* = 0.003). Nitrate (Hedges' *g* = -0.07, 95% CI -0.493-0.354, *p* = 0.748), BCAAs (Hedges' *g* = 0.11, 95% CI -0.418-0.631, *p* = 0.692), tyrosine (Hedges' *g* = 0.11, 95% CI -0.386-0.612, *p* = 0.658) and Eurycoma longifolia Jack (Hedges' *g* = 0.00, 95% CI -0.800-0.800, *p* = 1.000) had a *trivial* non-significant effect. Polyphenols (Hedges' *g* = 0.27, 95% CI -0.532-1.078, *p* = 0.506) had a *small* non-significant positive effect and caffeine (Hedges' *g* = 0.82, 95% CI 0.433-1.202, *p* < 0.001) had a *large* significant positive effect. Vitamin E (Hedges' *g* = -0.23, 95% CI -0.889-0.423, *p* = 0.487) had a *small* non-significant negative effect, creatine (Hedges' *g* = -0.63, 95% CI -1.507-0.256, *p* = 0.164) had a *medium* non-significant negative effect and taurine (Hedges' *g* = -0.96, 95% CI -1.855- -0.069, *p* = 0.035) had a *large* significant negative effect. Sub-group analysis of exercise modality demonstrated a non-significant effect of supplementation on end T_{core} (*p* = 0.231). As shown in Figure 4.5, the use of any supplement had a *trivial* non-significant effect on TTE (Hedges' *g* = -0.03, 95% CI -0.417-0.355, *p* = 0.875) and IST (Hedges' *g* = 0.16, 95% CI -0.641-0.963, *p* = 0.694), but had a *small* significant positive effect on TT end T_{core} (Hedges' *g* = 0.40, 95% CI 0.093-0.699, *p* = 0.010).

Sub-group analysis demonstrated a non-significant effect of the different supplement categories on submaximal T_{core} (*p* = 0.599). Nitrate (Hedges' *g* = -0.02, 95% CI -0.517-0.482, *p* = 0.945), BCAAs (Hedges' *g* = -0.05, 95% CI -0.580-0.474, *p* = 0.844), taurine (Hedges' *g* = 0.06, 95% CI -0.777-0.895, *p* = 0.890) and vitamin E (Hedges' *g* = 0.00, 95% CI -0.653-0.653, *p* = 1.000) had a *trivial* non-significant effect. Caffeine (Hedges' *g* = 0.49, 95% CI 0.090-0.894, *p* = 0.016) had a *small* significant positive effect. Tyrosine (Hedges' *g* = 0.34, 95% CI -0.165-0.846, *p* = 0.187) and polyphenols (Hedges' *g* = 0.21, 95% CI -0.590-1.016, *p* = 0.603) had a *small* non-significant positive effect. Creatine (Hedges' *g* = -0.27, 95% CI -1.136-0.586, *p* = 0.532) had a *small* non-significant negative effect. Sub-group analysis of exercise modality also demonstrated a non-significant effect of supplementation on submaximal T_{core} (*p* = 0.070). As shown in Figure 4.5, the use of any supplement had a *trivial* non-significant effect on TTE (Hedges' *g* < 0.01, 95% CI -0.281-0.278, *p* = 0.991), but had a *small* significant positive effect on TT submaximal T_{core} (Hedges' *g* = 0.37, 95% CI 0.082-0.654, *p* = 0.012).

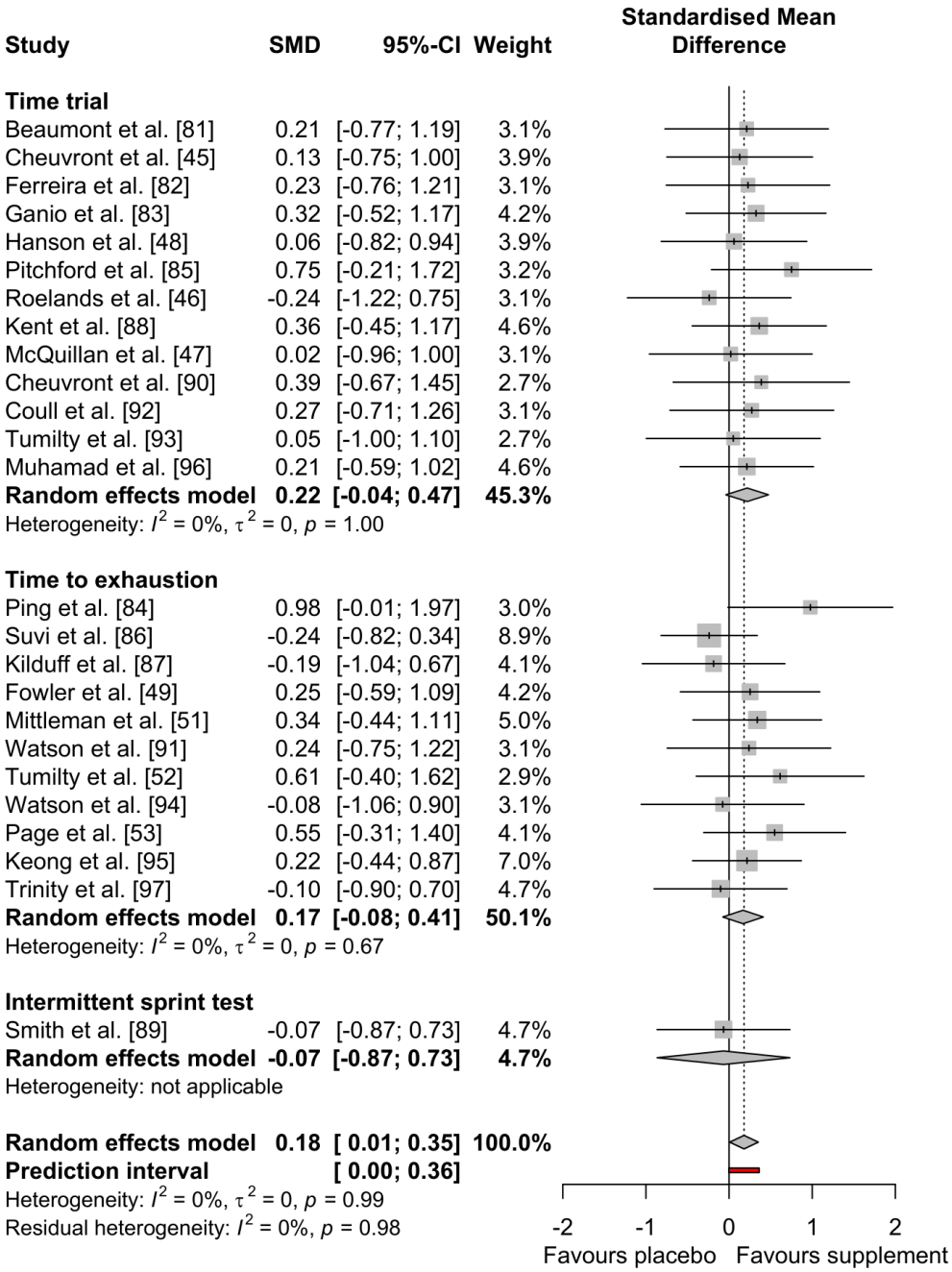


Figure 4.4. Effect of dietary supplementation on exercise performance by exercise modality.

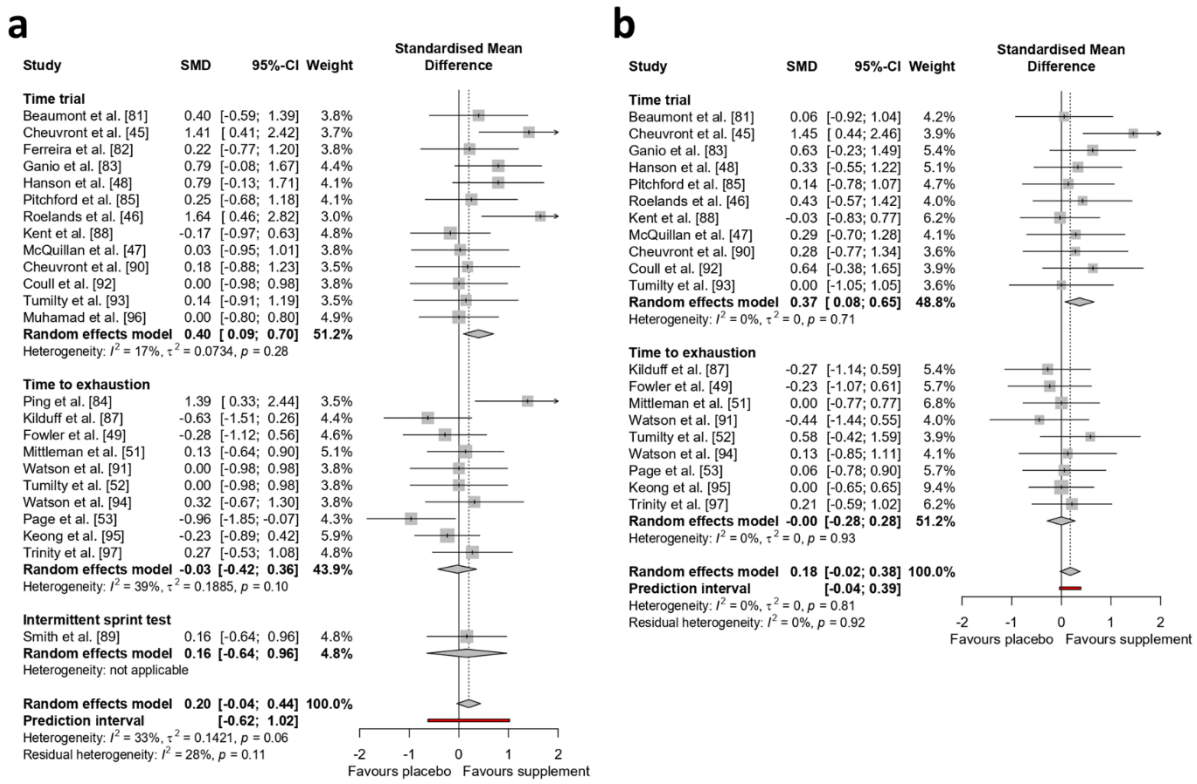


Figure 4.5. Effect of dietary supplementation on (a) end core temperature and (b) submaximal core temperature by exercise modality.

4.4.5. Meta-regression

Across the three meta-analyses, there was only one moderating effect: that of exercise before the performance protocol (exercise *vs* no exercise) on submaximal T_{core} responses (p = 0.039; Table 4.2). Otherwise, there were no significant moderating effects of any variables on the outcome of exercise performance and end T_{core} or submaximal T_{core} responses (Table 4.2).

Table 4.2. Meta-regression outcomes.

Moderator	Exercise performance	End core temperature response	Submaximal core temperature response
Training status	β = -0.021, p = 0.907 (n = 25)	β = 0.095, p = 0.707 (n = 24)	β = -0.084, p = 0.692 (n = 20)
Heat acclimation status	β = 0.247, p = 0.329 (n = 18)	β = 0.119, p = 0.770 (n = 17)	β = -0.139, p = 0.660 (n = 15)
Hydration status	β = -0.153, p = 0.783 (n = 16)	β = -0.005, p = 0.994 (n = 16)	β = -0.070, p = 0.909 (n = 12)
Fluid ingestion during exercise	β = 0.004, p = 0.983 (n = 22)	β = 0.222, p = 0.495 (n = 21)	β = -0.082, p = 0.751 (n = 17)
Fed *vs* fasted state	β = 0.062, p = 0.763 (n = 19)	β = -0.076, p = 0.819 (n = 18)	β = -0.064, p = 0.793 (n = 15)
Acute heat exposure beforehand	β = -0.144, p = 0.416 (n = 25)	β = 0.384, p = 0.113 (n = 24)	β = 0.363, p = 0.082 (n = 20)
Exercise beforehand	β = -0.183, p = 0.312 (n = 25)	β = 0.421, p = 0.089 (n = 24)	β = 0.449, p = 0.039 (n = 20)
Duration of performance protocol	β = 0.002, p = 0.532 (n = 24)	β < 0.001, p = 0.919 (n = 23)	β = -0.004, p = 0.152 (n = 19)
Total duration of exercise	β = 0.002, p = 0.491 (n = 24)	β = 0.004, p = 0.247 (n = 23)	β < 0.001, p = 0.952 (n = 19)

4.4.6. Risk of bias

The studies included had a generally ‘low’ or ‘unclear’ risk of bias, with all but three studies not stating randomisation procedures (Fowler et al., 2020; Page et al., 2019; Smith et al., 2019), and two studies not adopting a blind design (Cheuvront et al., 2004; Watson et al., 2012). Allocation concealment was ‘unclear’ in all studies (Figure 4.6). There were no outliers detected and Egger’s test showed that there was no publication bias in the exercise performance meta-analysis (p = 0.053). Several outliers (Page et al., 2019; Roelands et al., 2011) were detected in the end T_{core} meta-analysis, owing to the large effects certain supplements appear to have on end T_{core} responses. Egger’s test indicated publication bias (p

= 0.015; Figure 4.7) and, therefore, Duval and Tweedie's Trim and Fill procedure was conducted, but no meaningful adjustments to the data were made. One outlier was detected in the submaximal T_{core} meta-analysis (Cheuvront et al., 2009), but no publication bias was found (p = 0.115).

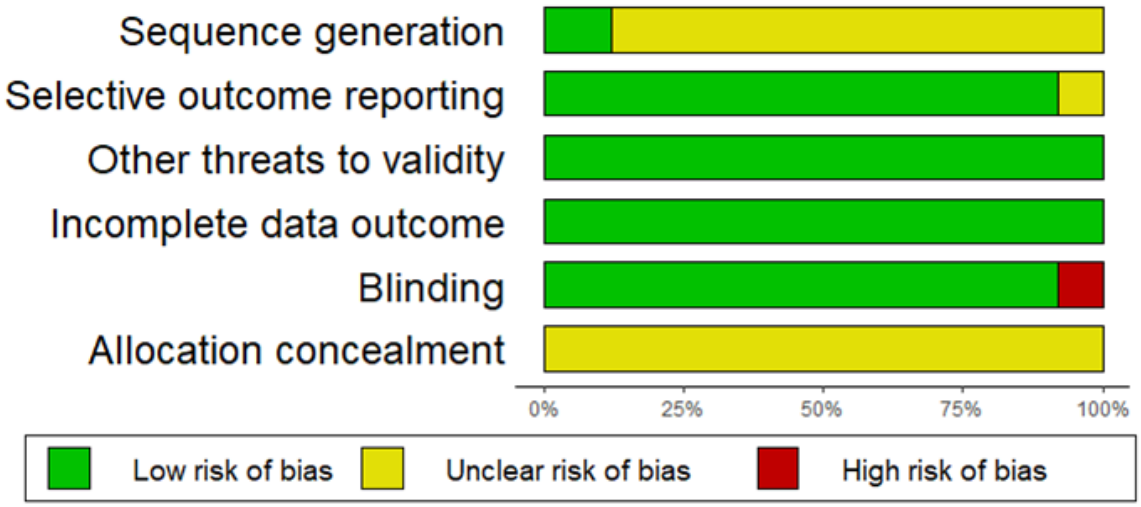


Figure 4.6. Risk of bias.

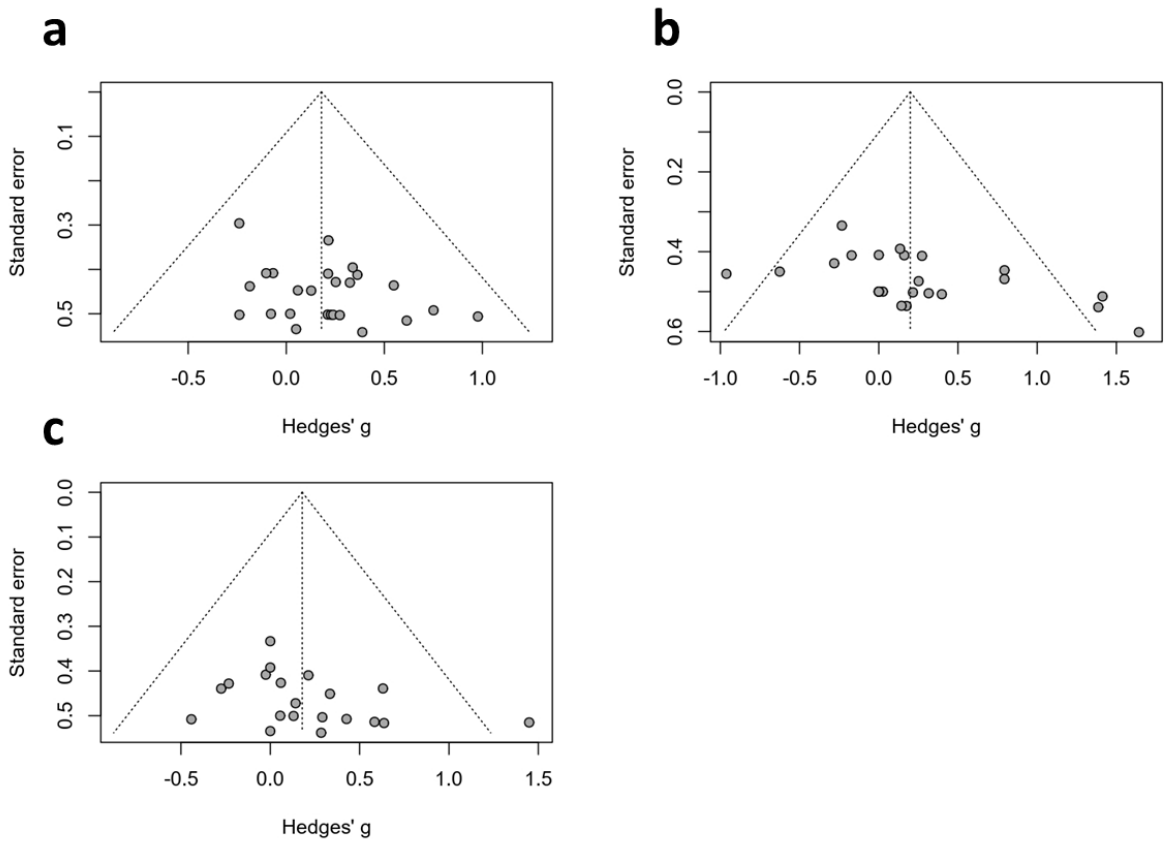


Figure 4.7. Publication bias for (a) exercise performance, (b) end core temperature and (c) submaximal core temperature.

4.5. Discussion

The main findings of the current meta-analyses were that dietary supplementation had a *trivial*, significant overall positive effect on endurance exercise performance in the heat (Hedges' g = 0.18, p = 0.042; Figure 4.2). The secondary sub-group analysis of exercise performance revealed no differences between supplements (p = 0.973); however, certain supplements, such as selected amino acids, demonstrated the greatest performance effect sizes in this analysis. Of particular note, caffeine (Hedges' g = 0.16, p = 0.263), creatine (Hedges' g = -0.19, p = 0.671) and nitrate (Hedges' g = 0.15, p = 0.490) had only a *trivial* and non-significant effect on endurance exercise performance in the heat, despite all of these supplements being recommended for athletes based on the strongest empirical evidence for performance enhancement in temperate conditions (Maughan et al., 2018; Thomas et al., 2016). The main findings of the T_{core} analyses were that, overall, dietary supplementation had a *small* but non-significant positive effect on end T_{core} (Hedges' g = 0.20, p = 0.104), and a *trivial* non-significant effect on submaximal T_{core} (Hedges' g = 0.18, p = 0.080; Figure 4.3). These results occurred irrespective of exercise duration, as demonstrated by the null effect of this moderating variable

(Table 4.2). The secondary sub-group analysis of end T_{core} demonstrated differences between supplements (p = 0.003), which was largely attributable to caffeine supplementation's thermogenic effect. This evidence was surprising, given that some mechanisms underpinning the thermoneutral ergogenic effects of caffeine and nitrate, in particular, should, theoretically, facilitate thermal balance and performance in hot environments. These include lowered metabolic cost of exercise (Lansley et al., 2011; Larsen et al., 2007; Larsen et al., 2010), peripheral vascular control (nitrate; Fujii, McGinn, Stapleton, et al., 2014a; McGinn et al., 2014; Welch et al., 2009) and improved central drive (caffeine; Davis et al., 2003). Therefore, the null findings presented herein have potentially profound implications for the use of these supplements in many performance scenarios, including major competitions or hazardous occupational settings. A possible explanation for this is that the effectiveness of otherwise established ergogenic dietary supplements is negated by the severity of hot environmental conditions. Regardless of the mechanistic reasons, these findings bring into question the depth of current understanding regarding supplementation in the heat and current recommendations should be tempered by this.

The analysis of T_{core} revealed that caffeine had a *large* (Hedges' g = 0.82, p < 0.001) and *small* (Hedges' g = 0.49, p = 0.016) significant positive effect on end and submaximal T_{core}, respectively. A significant rise in T_{core} across exercise stages will deplete available heat storage capacity, leading to earlier onset of hyperthermic symptoms and reduced exercise performance (González-Alonso et al., 1999). This could explain the lack of an overall ergogenic effect for caffeine. Several papers have highlighted caffeine's thermogenic effects (Cheuvront et al., 2009; Hanson et al., 2019; Roelands et al., 2011), but none have directly linked this to negative performance outcomes. Therefore, the current meta-analytic approach was necessary to identify this important trend across studies. Caffeine's effects are chiefly exerted via antagonism of centrally-located adenosine receptors, which act to increase the amount of circulating dopamine in the brain, as its release is inhibited by the binding of adenosine (Davis et al., 2003). The inhibition of the reuptake of dopamine has been shown to increase T_{core} (Watson et al., 2005) and, therefore, a greater dopamine concentration in the brain following caffeine administration could explain the increase in T_{core} demonstrated in the caffeine trials across studies. The oxygen uptake ($\dot{V}O_2$) response to exercise, at given exercise intensities, has also been reported to increase following caffeine ingestion compared to placebo, indicating increased metabolic heat production (Bell & McLellan, 2002), which further supports this observation. Irrespective of any potential performance benefits, a supplement that increases T_{core} when exercising in the heat could have potentially harmful effects. Given that heat illness during endurance events in hot environments is common and

presents a risk to sports (Howe & Boden, 2007) or tactical personnel (Armed, 2017; Bricknell, 1996; Cox et al., 2016), such outcomes should be more clearly recognised in dietary guidance.

In the current meta-analysis, a *trivial,* non-significant negative effect for polyphenols (Hedges' g = -0.10, p = 0.802), a supplement with known anti-oxidative properties was also found. While a *small* positive effect was found for the other anti-oxidants, Eurycoma longifolia Jack (Hedges' g = 0.21, p = 0.603) and vitamin E (Hedges' g = 0.22, p = 0.520), there were no significant differences found herein or between the supplementation and placebo groups in the original research articles. Anti-oxidants are thought to delay fatigue by removing damaging reactive oxygen species (ROS) from the muscle, and, therefore, counteracting exercise-induced oxidative stress (Powers et al., 2004). It was somewhat unanticipated that anti-oxidants did not improve endurance exercise in the heat, since thermal stress exacerbates oxidative stress due to increased ROS production in such conditions (McAnulty et al., 2005). A recent meta-analysis concluded that anti-oxidants have a moderate benefit to exercise performance in temperate conditions (Somerville et al., 2017); however, findings from individual studies remain equivocal. Studies reporting a considerable favourable effect on exercise performance administered a supra-physiological dose of *n*-acetylcysteine - a free radical scavenger - by intravenous infusion (McKenna et al., 2006; Medved, Brown, Bjorksten, & McKenna, 2004; Medved, Brown, Bjorksten, Murphy, et al., 2004). These findings are not supported by the majority of studies using oral anti-oxidant supplementation (Askari et al., 2013; Bigelman et al., 2010; Braakhuis et al., 2014; Cureton et al., 2009; Kang et al., 2012; Nieman et al., 2009; Scholten et al., 2015; Scribbans et al., 2014; Skarpañska-Stejnborn et al., 2008; Utter et al., 2009), with only a limited number finding a performance benefit (Davis et al., 2010; MacRae & Mefferd, 2006; Nieman et al., 2010; Roberts et al., 2015; Toscano et al., 2015). It is possible that the dose and method of administration observed in the studies included in the current analysis were insufficient to elicit an ergogenic effect. In response to the current findings, further investigation into supplements conferring anti-oxidative effects in hot conditions is certainly warranted.

The supplements with the greatest ergogenic effect on exercise performance in the heat were amino acids, with BCAAs (Hedges' g = 0.32, p = 0.232) and tyrosine (Hedges' g = 0.21, p = 0.404) having a *small* non-significant effect, and taurine (Hedges' g = 0.55, p = 0.209) having a *medium* non-significant effect. While non-significant overall, the effects of amino acids on exercise performance should not be discounted. Collectively, these supplements demonstrated the largest effect sizes, but there is currently insufficient evidence to recognise a significant effect. Interestingly, these are supplements with either equivocal or incomplete evidence for eliciting performance benefits in a thermoneutral environment (Chinevere et al., 2002; Davis et al., 1999; Negro et al., 2008; Strüder et al., 1998; Sutton et al., 2005; Van Hall

et al., 1995; Waldron et al., 2018a). The mechanism of action by which these amino acids provide an ergogenic effect is not fully understood, but reduced central fatigue is commonly ascribed to the ergogenic effects of BCAAs and tyrosine (Newsholme & Blomstrand, 2006; Tumilty et al., 2011). This theory suggests that a rise in plasma free fatty acid concentration due to prolonged exercise leads to tryptophan being displaced from albumin (Newsholme & Blomstrand, 1995). Consequently, the plasma concentration of unbound, free-tryptophan increases, resulting in greater transport across the blood-brain barrier and subsequent synthesis of serotonin (Newsholme & Blomstrand, 2006). This, in turn, causes lethargy, loss of drive, reduced motor unit recruitment and, ultimately, fatigue (Davis et al., 2000; Newsholme, 1987). Amino acids, such as BCAAs and tyrosine are thought to compete with tryptophan for transport across the blood-brain barrier, thus limiting its entry into the central nervous system, reducing the rate of serotonin synthesis and delaying fatigue (Blomstrand et al., 1991; Bongiovanni et al., 2010). Tyrosine is also a dopamine pre-cursor and dopamine plays a large role in increasing arousal, motivation and motor control (Nestler et al., 2001). Therefore, increased dopaminergic activity in the brain due to greater tyrosine concentrations may also delay fatigue, as well as increasing activation of motor pathways (Davis & Bailey, 1997). It is logical that these mechanisms could offset hyperthermic fatigue, as reduced central drive is observed during advanced heat stress, more so than during exercise in temperate conditions (Nybo, 2008; Nybo et al., 2011). However, while an overall positive effect of both BCAAs and tyrosine on performance within the current meta-analysis was demonstrated, the results of individual studies were inconsistent. The reasons for this are unclear, as while the exercise protocols, dosages (for BCAAs) and timings of ingestion differed slightly between studies, there were no apparent relationships between these variables and performance outcomes. Additional research is necessary to investigate this further.

Taurine, a sulphur containing amino acid, had the largest, albeit non-significant, effect on exercise performance in the heat of any of the supplements and also had a *large* significant negative effect on end T_{core} (Hedges' g = -0.96 p = 0.035). This suggests that taurine exerts a thermoregulatory effect that reduces T_{core}. Page et al. (2019) demonstrated that taurine increased sweating onset and rate, which might explain the improved thermal balance. These effects, in combination with taurine's capacity to enhance vasodilation (Sun et al., 2016), could facilitate both evaporative and dry heat transfer during exercise, delaying the rise in T_{core} and hyperthermic fatigue. In the animal model, central infusion of taurine, a GABA agonist, has been shown to reduce T_{core} in a dose-dependent manner (Frosini et al., 2003). Increased exogenous supply via oral supplementation could, therefore, offset the lower concentrations of GABA and taurine in hypothalamic nuclei following their heat stress-induced release (Frosini et al., 2000; Sharma, 2006). It should be stated that only one study (Page et al., 2019) has

been conducted regarding the effect of taurine supplementation on exercise performance in the heat and, therefore, further research needs to be conducted for corroboration and further mechanistic insight.

The secondary sub-group analysis of exercise modality (TT, TTE and IST) demonstrated no effect of supplementation on endurance exercise performance, or T_{core}. However, dietary supplementation did affect TT performance end T_{core} and submaximal T_{core}. A possible explanation for this is that the TTs included in the current analysis were generally performed at higher intensities, which is likely to elicit greater metabolic heat production and subsequent T_{core} responses. Only one of the meta-regression analyses performed was significant, where pre-trial exercise moderated (increased) the submaximal T_{core} outcome. This was anticipated because prior exercise may have already raised T_{core} to some degree, thus increasing submaximal T_{core}. Collectively, these results indicate that the overall thermogenic effect of dietary supplements (driven largely by caffeine) could be exacerbated by performing TTs or by performing pre-trial exercise. This could be important for athletes performing in the heat, where TT race formats are common and are often preceded by a warm-up activity (Hajoglou et al., 2005; Ückert & Joch, 2007). Close monitoring of body temperature and other signs of heat strain might therefore be important if selected supplements are orally ingested by athletes in hot TT races, alongside reduced intensity or duration of warm-up activities.

All candidate moderators, such as heat acclimation, training, hydration status, fluid ingestion during the trial and fed *vs* fasted state, did not affect exercise performance or T_{core} responses to the supplements. For heat acclimation status and hydration status, this is likely due to the majority of papers mandating the recruitment of non-heat acclimated and hydrated participants. Mixed with the homogenously low effect found among most supplements in the heat, there was likely to be insufficient variation of data to establish a relationship between these variables and their effects. However, there was less consistent control of variables, such as training status, fluid ingestion during the trial, and fed *vs* fasted state, yet no moderating effect was found, indicating that these could not explain the variance found in any supplements effect. On the basis of the current analysis, the effects reported could not be explained by candidate moderators but it would be useful to understand the efficacy of the most ergogenic supplements among participants of different training or heat acclimation statuses, given the effect of these processes on the acclimated phenotype (Ravanelli et al., 2018) and the likelihood of this scenario in real-world athletic or occupational settings.

There are still a number of factors not fully investigated which provide limitations to the current understanding of dietary supplementation for endurance exercise performance in the heat. The majority of papers used acute supplementation regimes and, therefore, the effect of

chronic supplementation on exercise performance in the heat (as well as safety and health) is still not well understood. Evaluation of this might be necessary for the more efficacious supplements observed here, such as taurine, and those with known benefits of chronic supplementation in thermoneutral conditions such as creatine, as this may elicit further effects. Similarly, the majority of exercise protocols were relatively short, with only nine trials exceeding one hour, thus limiting the current understanding of certain supplements on prolonged exercise in the heat. This is particularly important because prolonged exercise increases the probability of heat-related illness (Armstrong et al., 2007), which is extremely common in some occupations, such as military settings (Nindl et al., 2013). Finally, there is a lack of 'real-world' tasks performed in the studies included in the current meta-analysis, as all but one of the studies were controlled laboratory-based investigations. Therefore, the current results need replicating in ecologically valid conditions, to establish their real-world effectiveness.

4.5.1. Conclusion

In summary, for the first time, the effect of dietary supplementation on endurance exercise performance in the heat has been evaluated. Supplements, such as caffeine and nitrate, which have the strongest empirical support for use in temperate conditions, lack sufficient data to support their use in the heat. Core temperature responses were also increased with caffeine supplementation, without any ergogenic benefit, which has potentially harmful health and performance consequences. Anti-oxidants also do not appear to provide a performance benefit in hot conditions. On the other hand, amino acids appear to provide a greater performance benefit during exercise in the heat but the effects were often statistically insignificant. Branched-chain amino acids offered the most consistent, yet *small*, performance effect, while taurine had both the greatest performance and thermoregulatory effect sizes of any of the supplements included in the current meta-analysis, albeit from a single study. Although further research is certainly needed, these supplements have potential to be effective for individuals exercising in hot environments. It appears that exercising in the heat significantly influences the efficacy of many dietary supplements, suggesting that findings from research conducted on certain supplements in thermoneutral conditions are not necessarily transferrable to other environmental conditions. As such, research regarding the ergogenic effect of many dietary supplements for exercise in the heat is warranted. Future research should focus on understanding the mechanistic reasons for caffeine's thermogenic effects and, conversely, the thermolytic effects of taurine. The inconsistent ergogenic effects of amino acids also require further investigation, as the efficacy of their use is uncertain based on the current evidence. Collectively, these findings indicate that current dietary supplementation

guidelines for exercise in hot environments must be adapted and require further detail for sports and tactical personnel.

5. Chapter Five – The effect of dietary supplements on resting and exercising core temperature and sweating thermoregulatory responses in hot environmental conditions: a meta-analysis and meta-regression.

5.1. Abstract

Background Dietary supplements are widely used among individuals exposed to hot environments, but it is currently unclear whether their consumption confers any thermoregulatory advantages or disadvantages. This raises the need to systematically evaluate their effects on key aspects of thermoregulation.

Objectives The objectives of the present review were two-fold: (1) To meta-analyse studies investigating the effects of dietary supplements on T_{core} and sweating thermoregulatory responses during rest and exercise in the heat. (2) To conduct meta-regressions to evaluate the influence of selected variables (e.g. training and heat acclimation status), which are known to influence thermoregulatory functioning, on T_{core} and sweating responses in the heat following dietary supplementation.

Methods Three databases (Pubmed, Scopus and SPORTDiscus) were searched in February 2023. After screening, 119 peer-reviewed articles were identified for inclusion within three separate meta-analyses: (1) end T_{core}; (2) whole-body sweat rate (WBSR); (3) LSR. The moderating effect of several variables were assessed via sub-analysis and meta-regression.

Results Overall, dietary supplements had *trivial* non-significant effects on end T_{core} (defined as T_{core} at the point of the highest thermal strain; Hedges' g = 0.001, p = 0.978), WBSR (Hedges' g = 0.022, p = 0.758) and LSR (Hedges' g = 0.004, p = 0.976) in the heat. There was no overall effect of the differing supplement types on WBSR (p = 0.510) and LSR (p = 0.864), despite taurine significantly increasing WBSR (n = 1, Hedges' g = 0.96, p = 0.035) and gamma-aminobutyric acid (GABA; n = 2, Hedges' g = -0.78, p = 0.036) and whey protein (n = 1, Hedges' g = -1.31, p = 0.006) significantly reducing the WBSR response. Primarily due to caffeine's *small* significant positive effect (n = 28; Hedges' g = 0.43, p < 0.001) and taurine's *large* (n = 1, Hedges' g = -0.96, p = 0.035) and oligonol's *medium* significant negative effects (n = 3; Hedges' g = -0.50, p = 0.014), end T_{core} was significantly affected by supplement type (p = 0.016),

Conclusion Dietary supplements, such as amino acids (e.g. taurine), anti-oxidants and anti-inflammatories (e.g. oligonol) conferred the greatest thermoregulatory benefits during heat exposure. Taurine ingestion in such conditions may lower heat strain, likely through its

augmentation of thermal sweating. Conversely, caffeine intake may be detrimental to health and pose the greatest risk in the heat due to its apparent thermogenic effect.

5.2. Introduction

Adult humans rely on eccrine sweat production to facilitate evaporative cooling and maintain thermal balance, particularly in hot and/or humid environments (high wet-bulb globe temperature [WBGT]; Gagge & Gonzalez, 1996; Wenger, 1972). In such conditions, evaporation represents the primary heat transfer avenue (Wenger, 1972) and, consequently, thermal sweating is a vital physiological mechanism to offset heat storage within the body at rest, and particularly during exercise or occupational work (Marino et al., 2000; Sawka & Young, 2006). If heat is not sufficiently dissipated from the body to the environment, positive heat storage ensues (uncompensable heat stress), leading to T_{core} rises (Cramer & Jay, 2016). This heat strain can cause heat exhaustion, heat stroke and even death in extreme scenarios, if left uncorrected (Liu et al., 2022; Székely et al., 2015).

Thermoregulatory capacity is largely determined by three primary modifiable factors: metabolic heat production, vasodilation (i.e. dry heat loss) and sweat rate (i.e. evaporative heat loss; Benzinger et al., 1961b; Gagge & Gonzalez, 1996; Wendt et al., 2007). Consequently, the ability to activate thermoregulatory defences and to tolerate exposure to hot environmental conditions can be improved, with sweating the primary manipulable pathway (Périard et al., 2016; Ravanelli et al., 2018; Wenger, 1972). In healthy individuals, thermal sweating occurs in response to elevated T_{core} and/or T_{sk} (Hammel & Pierce, 1968; Werner, 1981). This temperature change is sensed by central and peripheral thermoreceptors, and processed in the preoptic area of the hypothalamus, stimulating sympathetic cholinergic neurons, which innervate eccrine sweat glands to stimulate sweat production through acetylcholine release (Shibasaki & Crandall, 2010). Various neuromodulators, such as catecholamines and NO, also have minor roles in eccrine sweating stimulation (Sato, 1993) and osmoreceptors, baroreceptors and muscle mechano- and metabo-receptors are thought to provide non-thermal stimulation for sweating (Shibasaki et al., 2003).

The threshold for sweating onset and the rate at which sweating occurs (thermosensitivity) can be chronically or acutely altered through manipulation of any of the above modifiable factors. For example, endurance training and heat acclimation regimes, are capable of lowering the oxygen cost of exercise at a given intensity and resting T_{core} but are notable in their capacity to accelerate sweating onset and increase sweat rate, alongside plasma volume and SkBF changes (Lorenzo et al., 2010; Périard et al., 2016; Poirier et al., 2016; Ravanelli et al., 2018; Rivas et al., 2017). In various ways, these physiological adaptations augment

avenues of heat transfer, control heat production and, ultimately, aid in maintaining thermal equilibrium during heat exposure. Given the importance of thermal sweating in achieving this, further understanding of the capacity for adaptation in sweating variables in response to various interventions is required.

More recently, the notion that dietary supplementation may offer thermoregulatory benefits or, alternatively, heighten the risk of heat illness when ingested in hot conditions has been considered (Jardine et al., 2023; Twycross-Lewis et al., 2016; Chapter Four). There are a number of motivations for individuals to consider dietary supplementation, such as ensuring adequate intake of certain nutrients, improving health and supporting specific physiological functions (Bailey et al., 2013; EFSA, 2024). Approximately 50% of US adults (Kantor et al., 2016) and between 15 to 41% of UK adults (Swan, 2016) report dietary supplement use, with only a quarter of users taking supplements that have been recommended by a healthcare professional (Ronis et al., 2018). Whilst such dietary supplements are not commonly consumed for the purpose of influencing thermoregulation, they may inadvertently affect it (Chapter Four). As the popularity of dietary supplements continues to rise in a world which is likely to experience evermore frequent, prolonged and intense heatwaves (Perkins-Kirkpatrick & Lewis, 2020), research is needed to better understand the potential thermoregulatory effects upon human health and performance. For example, the amino acid taurine may be ingested to rectify taurine deficiency and has a number of potential health benefits, such as anti-oxidative and anti-hypertensive effects (Schaffer et al., 2014; Sun et al., 2016). More recently, taurine has been reported to increase sweating rate (a key modifiable heat dissipation pathway) by approximately 13% and, concomitantly, reduce T_{core} compared to placebo in the heat (Page et al., 2019). Another dietary supplement, creatine, is not typically considered to be an aid to offset hyperthermia and is commonly taken to improve high-intensity exercise performance (Branch, 2003) and has also been recognised as a dietary strategy to enhance cognitive function (Avgerinos et al., 2018; Rawson & Venezia, 2011; Roschel et al., 2021). However, a review of its thermoregulatory effects highlighted that supplementation may be beneficial during exercise in high ambient temperatures due to its effects on fluid balance (Twycross-Lewis et al., 2016). Additionally, a recent meta-analysis established that pre-exercise hyperhydration with glycerol and/or creatine supplementation lowered T_{core} after constant work exercise in both thermoneutral and hot conditions (Jardine et al., 2023).

Dietary nitrate (NO_3^-) has a key role in supporting blood pressure reduction and vaso-protective activity (Benjamim et al., 2022; Lara et al., 2016; Li et al., 2020; Siervo et al., 2013) and can be supplemented among those with cardiovascular disease (Jackson et al., 2018), hypertension (Benjamim et al., 2022) or, indeed, heathy individuals to elicit such benefits (Zhang et al., 2023). There has also been considerable research to support its role in

endurance exercise enhancement (Gao et al., 2021; McMahon et al., 2017), yet there is a lack of evidence regarding any ergogenic roles in the heat (Chapter Four). This is surprising, as there is a plausible mechanistic basis for thermoregulatory enhancement following ingestion of dietary nitrate and L-arginine, as both are known to improve NO bioavailability (Lundberg et al., 2008; Moncada & Higgs, 1991). Specifically, NO bioavailability could have direct and indirect effects on eccrine sweat gland and microvascular function (Fujii et al., 2016; Stapleton et al., 2014a). Additionally, anti-oxidants such as polyphenols, may support thermoregulation through protection of NO against oxidative destruction, thereby improving its bioavailability (Ignarro et al., 2006) and enhancing or preserving peripheral vasodilation. However, given that body fluid loss, and secondary hypovolemia, is accelerated in the heat (Sawka et al., 1984), the reported reductions in blood pressure following supplementation with NO donors (Benjamim et al., 2022) could increase the risk of acute hypotension, particularly in the post-exercising state (Halliwill, 2001). Other supplementation strategies, such as oral administration of menthol, appear to augment human performance in the heat and lower thermal perception (Jeffries & Waldron, 2019) via non-thermal activation of cold sensory pathways but without affecting body temperature (Andersen et al., 2014). This effectively decreases the perceived sensitivity to changes in body temperature, which may be a disadvantage or dangerous to human health in advanced states of hyperthermia. It is, therefore, necessary to comprehensively review these supplements to understand the magnitude of effects they may have on thermoregulatory responses.

Whilst less commonly supplemented for health-related reasons among the general population (Moore et al., 2020), amino acids have a wide variety of biological roles, and can be supplemented to account for age related decline in lean muscle mass (Børsheim et al., 2008). For example, tyrosine is used to enhance cognitive function (Hase et al., 2015; Hensel et al., 2019), whilst BCAAs have been reported to alleviate skeletal muscle damage and soreness following exhaustive and resistance exercise (Fedewa et al., 2019; Rahimi et al., 2017). Furthermore, among those with potential acute or chronic tyrosine deficiency, dietary supplementation may offer greater availability to maintain catecholamine levels, which are important for sympathetic vasoconstrictive effects on the subcutaneous vasculature (Lang et al., 2020) and may impact upon both dry and evaporative thermoregulatory defences. Given that both tyrosine and BCAAs may compete for the same blood-brain-barrier transporters, coupled with their wider roles in neurotransmitter biosynthesis pathways (Fernstrom, 1981; Pardridge, 1998; Suryawan et al., 1998), sufficient balance of both supplements may be important during heat exposure. In the previous meta-analysis within this thesis, the use of orally administered tyrosine or BCAAs (used separately), were capable of enhancing endurance exercise performance in the heat, but there was no effect on sub-maximal or

maximal T_{core} responses (Chapter Four), thereby questioning their thermoregulatory role. Whilst many of the above-mentioned supplements are used more modestly across the population (Mishra et al., 2021; Moore et al., 2020), caffeine features in the daily intake of approximately 80 to 85% of people globally (Heckman et al., 2010; Mitchell et al., 2014) and is a prominent dietary supplement among athletes (Del Coso et al., 2011). However, caffeine has been reported to increase T_{core} when ingested before or during exercise in the heat (Chapter Four), but its effects in the resting state have not been evaluated meta-analytically. Given the high prevalence of caffeine consumption, mixed with the understanding of its cardiometabolic side-effects (de Souza et al., 2022; Zulli et al., 2016), this perhaps places one of the greatest risks to the general population when consumed in the heat. Collectively, it is apparent that supplementing the diet with some substances, could have implications for thermoregulatory capacity, and further research is required to understand the consistency and magnitude of effects reported across the empirical literature.

Based on the evidence to date, a systematic evaluation of the effect of all dietary supplements on the primary modifiable thermoregulatory process of sweating, and subsequent T_{core} responses, is warranted. This is necessary to provide clarity on the potential mechanisms by which various supplements are affecting thermal balance during rest and exercise. This impact has not previously been fully considered, and there remains limited official guidance on dietary supplement intake for those exposed to thermally stressful conditions, such as athletes (Collins et al., 2020; Maughan et al., 2018; Thomas et al., 2016), and military personnel (United States Army Field Manual 7-22) or, indeed, the general public (WHO, 2012). The UK commander's guidelines (JSP 375) do provide some information regarding supplement intake for military personnel in scenarios of increased heat stress risk, such as to avoid stimulants and diuretics. However, this advice is not specific to individual supplements and their potential effects, particularly in thermally stressful environments. Given the range of effects that different supplements appear to have on T_{core}, at least in the exercising state in the heat (Chapter Four), coupled with the clear lack of specific guidance on this topic, a comprehensive evaluation of the collective evidence is an important step in developing an evidence-based understanding of the benefits or risks associated with using dietary supplements in hot conditions.

The aims of the current meta-analysis were to investigate the effects of all known orally administered dietary supplements on resting and exercising T_{core} and sweating thermoregulatory responses in the heat. The effect of rehydration solutions, such as electrolytes, on thermal sweating have been thoroughly evaluated (Périard et al., 2021; Sawka et al., 1984; Sawka & Montain, 2000) and were not replicated here; however, a number of factors were considered as moderators of T_{core} and sweating responses, such as hydration status among participants in studies evaluating dietary supplementation in the heat (Sawka et

al., 1998; Sawka et al., 2001). Likewise, training and acclimatisation/acclimation status (Ravanelli et al., 2018), protocol (rest *vs* exercise) and exercise intensity were considered to potentially impact thermoregulatory sweating and T_{core} (Cramer & Jay, 2014; Gagnon et al., 2013; Chapter Six). Environmental conditions, such as WBGT (and/or heat stress index; and vapour pressure) will also influence the ability to evaporatively cool (Che Muhamed et al., 2016; Cramer & Jay, 2019). Therefore, to evaluate the effects of dietary supplements on thermoregulation in the heat, these factors were considered as potential moderating variables and formed part of a secondary meta-regression analysis.

5.3. Methods

5.3.1. Search strategy

All of the available literature that investigated the effects of dietary supplementation on thermoregulatory responses, including sweating, in a hot environment was searched and obtained according to the PRISMA guidelines, with a predetermined search strategy (Moher et al., 2015). Medical subject heading (MeSH) terms were active during the searches. There was no limit on the status, date or language of the publication. The final Boolean searches were performed in PubMed, SPORTDiscus (EBSCO) and Scopus on 6th February 2023. The search terms used were '(dietary supplements OR dietary supplementation OR nutritional supplements OR nutritional supplementation OR supplements OR supplementation OR ergogenic OR ergogenic aids OR nutraceuticals OR amino acids OR anti-oxidants OR vitamins OR minerals OR stimulants OR herbs OR herbal) AND (heat OR temperature OR sweat OR sweating OR sweat response OR sweating response OR sudomotor OR body temperature regulation OR thermoregulation OR thermoregulatory OR heat loss OR cooling OR evaporative OR evaporation OR thermal stress OR heat stress OR hyperthermia OR hyperthermic)'. As there is no *a-priori* list of dietary supplements that effect thermal balance, no supplements were searched individually by name. Two investigators (JP and MW) verified the search terms and the accuracy of the returned results. 'Other sources' were also identified, such as through social media (Twitter or 'X').

5.3.2. Study selection

Any duplicates were removed, and titles and abstracts were screened for inclusion by two investigators (JP and MW), in accordance with an agreed inclusion criteria. The single paper retrieved which had been published in a language other than English was translated digitally using two separate translation software programs; Google Translate and DeepL Translator

(DeepL GmbH, Cologne, Germany). The reference lists of the initial papers were reviewed independently by two investigators (JP and MW). The remaining articles were then assessed separately (and without influence) by JP and MW against the inclusion and exclusion criteria. There was 100% agreement in study selection between the two reviewers. Papers were required to have been published in a peer-reviewed journal as original research articles with a cross-over, randomised control trial, an intervention or an independent groups design. They must also have included a control or placebo group, and participants were required to be healthy adults (≥ 18 years). To be included in this analysis, the studies must have: (1) administered a dietary supplement (by the definition below); (2) been conducted in an ambient dry-bulb temperature of ≥ 30 °C or WBGT ≥ 20 °C or small ranges up to those temperatures in either a laboratory or field setting. A WBGT of ≥ 20 °C was considered to provide sufficient heat stress, even when dry-bulb temperature was < 30 °C (Budd, 2008). Of the remaining papers, 71 were removed for the reasons outlined in Figure 5.1, primarily that they included supplements that were: a drug; not orally administered; a macro-nutrient or a rehydration solution (e.g. electrolytes or a supplement with a mechanism of action considered to be directly related to hydration). Other reasons were the absence of measures of T_{core} and the sweating response or environmental issues.

A dietary supplement was defined by adapting the IOC position statement (Maughan et al., 2018) and the European Food Safety Authority statement (EFSA, 2024): a non-food, non-pharmacological, food component, nutrient or non-food compound that is purposefully orally ingested in addition to the habitual diet, for its nutritional or physiological effects. This may be to maintain sufficient intake of certain nutrients, correct deficiencies, or support physiological functions, including thermoregulatory responses to the heat. The supplement is not being consumed for its calorific value, its effects on hydration (the mechanism of action is not through rehydration) and is not an energy drink. Ingestion of the supplement is also recognised to be legal as per the Misuse of Drugs Act 1971 (U.K. Government, 1971) and is not on the World Anti-Doping Association's prohibited substances list (WADA, 2023).

5.3.3. Data extraction and quality assessment

Data were extracted and entered into a custom-designed Microsoft Excel spreadsheet. Extracted data included: (1) characteristics of the sample (sex, age, health, training and heat acclimation/acclimatisation status); (2) study design; (3) supplement, dose and timing of intake; (4) fluid intake before and during exercise i.e. hydration status; (5) environmental conditions (temperature and humidity); (6) trial type i.e. exercise type or rest and length; (7) end T_{core} (rectal, gastrointestinal, oesophageal or tympanic); and (8) bias. Risk of bias was

assessed independently by two investigators (JP and MW) according to the Cochrane collaboration guidelines (Higgins et al., 2019). Where details of the study were unclear, the authors of the relevant papers were contacted for specific information or to clarify the method that was used. There was 100% agreement between the investigators concerning the outcome of this quality assurance procedure, hence, it was not considered necessary to include a third independent reviewer. There were three outcome measures for this meta-analysis: (1) T_{core} reported at the end of the trial, the end of the exercising portion of the trial or at the point of the highest thermal strain, hereafter referred to as 'end T_{core}'; (2) WBSR across the trial or exercising portion of the trial; and (3) LSR reported at the end of the trial or at the point of the highest thermal strain.

5.3.4. Statistical analysis

Data were extracted from the qualifying papers in the form of a mean, standard deviation (SD) and sample size (*n*) for the meta-analysis. Publicly available software (WebPlotDigitizer, Version 4.3) was used to extrapolate any unreported values from the figures to mean and SD data. Where data were expressed as mean and standard error (SE or SEM) or CI, they were converted to mean and SD. Authors of the original research articles were contacted for any missing data; however, if this was not accessible, they were imputed using the sample pooled SD from similar included studies (Furukawa et al., 2006). For selected study designs (i.e. intervention studies with pre-post supplementation), the post-intervention values were extracted as the outcome measures for the 'supplementation condition' and the pre-intervention values as the 'placebo or control condition' (Higgins et al., 2019). For cross-over trials (within-subject) or independent designs, the outcome measures for the supplementation condition were considered against the placebo or control condition. Standardised mean difference (SMD) was used to compare the results between studies utilising different protocols and measures. End T_{core} outcome data were reported as end T_{core} (°C) or rate of rise (°C·h^{-1}) of T_{core}. Mean, maximum, peak and mean body temperature were also included if end T_{core} data were not provided. Whole-body sweating response outcome data were reported as WBSR (mL·min^{-1}) and body mass change (%). Outcome data representing WBSL (i.e. body mass or sweat loss and body mass change), reported in absolute L, mL, kg or g were converted to WBSR (mL·min^{-1}) using trial length data and WBSR reported in L·h^{-1} or mL·h^{-1} were directly converted to mL·min^{-1}. LSR outcome data reported in nL·min^{-1}, were converted to mg·cm·min^{-1} and reported as such.

Three meta-analyses were conducted, one for each outcome measure. These were performed in RStudio (R Core Team; Harrer et al., 2019) and included 125, 100 and 10 comparison

groups for the end T_{core}, WBSR and LSR meta-analyses, respectively. Not all studies reported T_{core} or a sweating response data, hence, they were excluded from the respective analyses. All data were analysed with a random-effects model, with heterogeneity assessed using the I^2 statistic. Outliers were detected using a function in RStudio and influence on analysis investigated. Publication bias was accounted for by funnel plots and conducting Egger's test and, subsequently, Duval and Tweedie's Trim and Fill procedure, when indicated (Rothstein et al., 2005). Any adjustments to the effect sizes based on this procedure are reported in the results. Hedges' *g* and 95% CIs were used to express SMD between dietary supplementation and placebo groups across studies. Sub-analysis of the different dietary supplements included were conducted for all three meta-analyses. Meta-regressions were also conducted to determine the effect of candidate moderators on end T_{core}, WBSR and LSR outcomes, as reported in each study: training status (highly trained *vs* recreationally active); heat acclimation status (heat acclimated *vs* non-heat acclimated); hydration status (euhydrated *vs* hypohydrated); fluid ingestion during exercise (fluid ingestion *vs* no fluid ingestion); duration of trial; WBGT; trial type (exercise *vs* rest); supplement dose (where sufficient no. of studies) and duration of supplementation (where applicable). The thresholds for the magnitude of effects were < 0.2, 0.2, 0.5 and 0.8 for *trivial, small*, *medium* and *large* effects, respectively (Rosenthal & Rosnow, 2008). Alpha (α) was set at $P \leq 0.05$ for all analyses.

5.4. Results

5.4.1. Study selection

The initial searches retrieved 37,641 articles, which were reduced to 37,140 after removal of duplicates. After further screening and removal of reviews, animal studies and other irrelevant papers, 167 articles remained. Searches of social media (Twitter or 'X'), additional databases and reference lists within the 167 papers provided 32 further papers. Of the 199 articles, 48 were removed based on their incomplete compliance with the inclusion criteria and a further 32 were removed due to having: no full-text available, duplicate data with another paper or no extractable data. This left 119 papers, of which 112, 89 and 9 papers were included in the end T_{core}, WBSR and LSR analyses, respectively (Figure 5.1). Fifteen papers had more than one comparison group and, therefore, one or more additional data sets were added to the analysis for each study. As these additional comparison groups shared participants, the sample size was reduced to mitigate any unit-of-analysis error, as per the Cochrane guidelines (Higgins et al., 2019). Three papers also included multiple comparison groups; however, as these did not share participants, they were included without sample size adjustment. One paper was included without addition of the duplicate end T_{core} data.

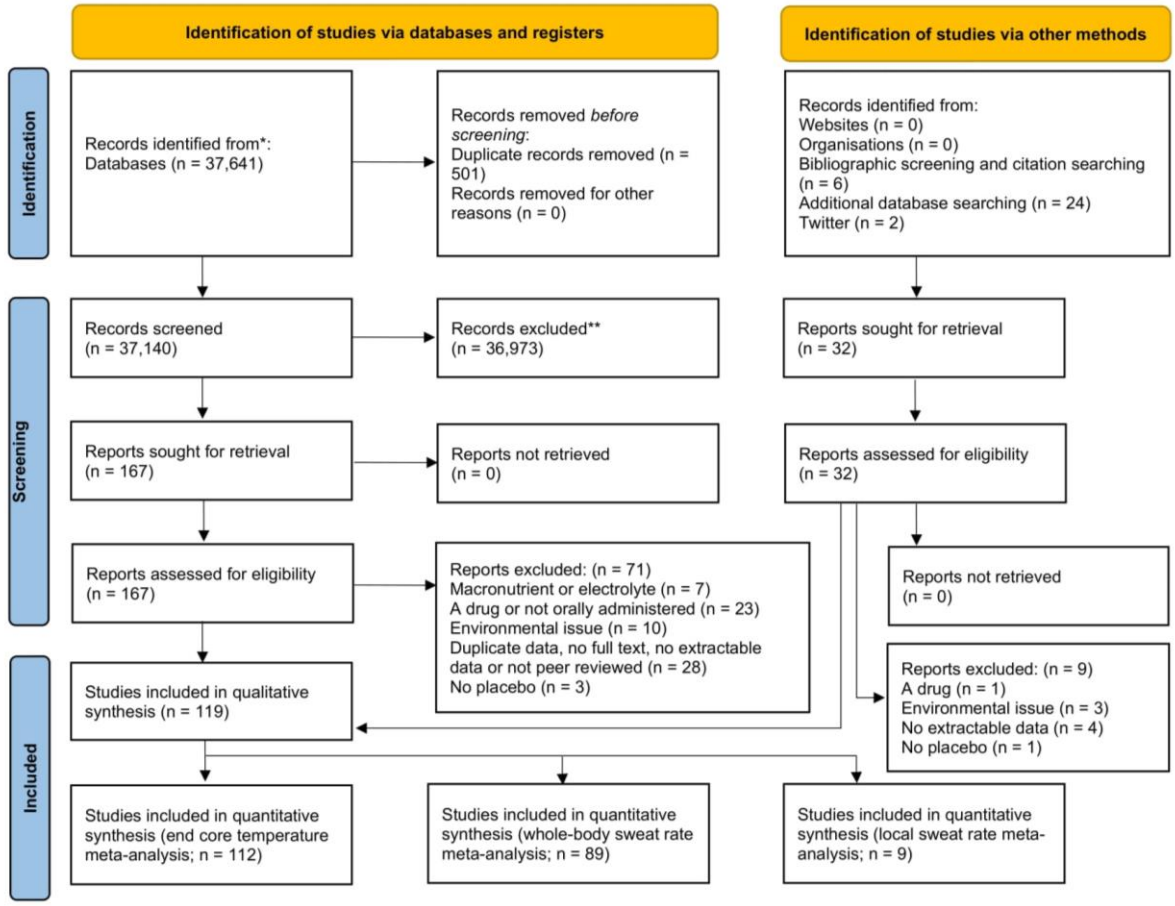


Figure 5.1. The process of study selection.

5.4.2. Study characteristics

The characteristics of the 119 included studies are summarised in Table 5.1. The studies included a total of 1,484 participants, comprising both males and females (males 91%; both males and females 9%) of varying training (highly trained 43%; recreationally active 39%; unreported 18%) and heat acclimation statuses (heat acclimated 14%; non-heated acclimated 36%; unreported 50%). A hundred and one studies were cross-over designs, 12 studies were an independent groups design, and 6 studies were pre-post interventions. Thirty-seven different types of dietary supplements or supplement combinations were included in varying doses (Table 5.1). These were a combination of acute doses (single day; *n* = 78; 66%) and chronic administration (≥ 2 days; *n* = 41; 34%). The trial types included were exercise (90%) and rest (10%). The measures of T_{core} were rectal (62%), tympanic (10%), oesophageal (9%), gastrointestinal (14%), oral (1%) and unreported (4%). The measures of body mass or sweat loss or sweat rate, representing WBSR were reported in L (7%), mL (7%), kg (17%) or g (3%),

$g \cdot m^{-2} \cdot h^{-1}$ (1%), % change (11%), $L \cdot h^{-1}$ (17%), $mL \cdot h^{-1}$ (1%) and $mL \cdot min^{-1}$ (7%) or where unreported (29%). Ambient dry-bulb temperature (mean 33.8°C; range 25 to 46.6°C), WBGT (mean 27.4°C; range 18.5 to 35.1°C) and RH% (mean 47%; range 12 to 80%) are reported herein. There were no adverse health-related events noted in any of the studies.

5.4.3. Meta-analysis

The results of the end T_{core} meta-analysis (n = 127) are reported in Figure 5.2. Overall, there was a *trivial* non-significant positive effect of all supplements on end T_{core} compared to placebo (Hedges' g = 0.001, 95% CI -0.097-0.100, p = 0.978). The I^2 statistic demonstrated 21.4% heterogeneity. The results of the WBSR (n = 101) and LSR (n = 10) meta-analyses are reported in Figure 5.3 and Figure 5.4, respectively. Overall, WBSR (Hedges' g = 0.022, 95% CI -0.121-0.169, p = 0.745) and LSR (Hedges' g = 0.004, 95% CI -0.257-0.265, p = 0.976) had a *trivial* non-significant increase with dietary supplementation compared to placebo, with 0% heterogeneity (I^2).

Table 5.1. Summary of studies included in the meta-analyses (n = 119).

Study	Design	Sample	Supplement, dose and timing	Temperature and relative humidity	Core temperature method	Trial type	Sweating measure
Beaumont & James (2017)	Double-blind, randomised, repeated-measures, placebo-controlled, cross-over	Healthy, recreationally active, non-heat acclimated males (*n* = 8). Age 22 ± 1 years	Caffeine 6 mg·kg^{-1} (60 min pre-exercise)	30°C 50% RH WBGT 24.6°C	Gastrointestinal every 5 min (ECT)	60 min cycling @ 55% W_{max} followed by 30 min TT	Sweat rate (L). Converted to WBSR (mL·min^{-1})
Cheuvront et al. (2009) A and B	Double-blind, randomised, placebo-controlled, cross-over	Healthy, physically active, moderately fit, non-heat acclimated males (*n* = 10). Age 23 (18-37) years	Caffeine 9 mg·kg^{-1} (timing not mentioned) A Quercetin 2000 mg (timing not mentioned) B	40°C 20-30% RH WBGT 28-30.1°C	Rectal every 5 min (ECT)	30 min cycling @ 50% $\dot{V}O_{2peak}$ followed by 15 min TT	Sweat rate (L·h^{-1}). Converted to WBSR (mL·min^{-1})
Cohen et al. (1996) A and B	Double-blind, randomised, placebo-controlled, cross-over	Healthy, heat acclimatised, competitive male (*n* = 5) and female (*n* = 2) runners (*n* = 7). Age 33.3 ± 9.2 years	Caffeine 5 mg·kg^{-1} (60 min pre-exercise) A Caffeine 9 mg·kg^{-1} (60 min pre-exercise) B	WBGT 24-28°C	Tympanic pre and post exercise (ECT)	21 km running TT	Body mass change (%).
Del Coso et al. (2009)	Double-blind, randomised, placebo-controlled, cross-over	Healthy, heat acclimated, endurance trained males (*n* = 7). Age 27 ± 1 years.	Caffeine 6 mg·kg^{-1} (45 min pre-exercise)	36.0°C 29.0% RH WBGT 26.7°C	Rectal every 10 min (ECT)	120 min cycling @ 63% $\dot{V}O_{2max}$	Sweat loss (L). Converted to WBSR (mL·min^{-1})
Dias et al. (2005)[1] A and B	Double-blind, randomised,	Healthy males (*n* = 59; 20 *vs* 20 *vs* 19)	Caffeine	37.7°C	Rectal (no T_{core} data reported)	90 min treadmill walking @ 5.6	Body-weight loss (kg).

	independent design	Age 21.6 ± 6.9 years	3 mg·kg^{-1} (6 x 3 mg·kg·d^{-1}; no timing mentioned) A Caffeine 6 mg·kg^{-1} (5 x 6 mg·kg·d^{-1}; no timing mentioned) B	56.3% RH WBGT 32.4°C		km·h^{-1} with a 5% incline	Converted to WBSR (mL·min^{-1})
Ely et al. (2011)	Double-blind, counter-balanced, placebo-controlled, cross-over	Healthy, moderately fit, non-heat acclimated males (*n* = 10). Age 23 (range 19) years	Caffeine 9 mg·kg^{-1} (90 min pre-exercise)	40°C 25% RH WBGT 29.1°C	Rectal every 5 min (reported mean body temperature; End mean body temperature)	30 min cycling @ 50% $\dot{V}O_{2peak}$	Sweat rate (L·h^{-1}). Converted to WBSR (mL·min^{-1})
Falk et al. (1990)	Double-blind, placebo-controlled, cross-over	Trained males (*n* = 7). Age 23.8 ± 2.4 years	Caffeine 5 mg·kg^{-1} (120 min pre-exercise) and 2.5 mg·kg^{-1} (30 min pre-exercise)	25°C 50% RH WBGT 20.3°C	Rectal (ECT)	Treadmill walking @ 70-75% $\dot{V}O_{2max}$ (speed 1.56 s^{-1} with a 22-kg backpack)	Water loss (mL). Converted to WBSR (mL·min^{-1})
Ferreira et al. (2005) A and B	Double-blind, randomised, placebo-controlled, cross-over	Well-trained, heat acclimated, male cyclists (*n* = 8). Age 23.9 ± 8.6 years	Caffeine 5 mg·kg^{-1} (60 min pre-exercise) A Caffeine 9 mg·kg^{-1} (60 min pre-exercise) B	30°C average, ranged from 28.5-32°C 71-78% RH WBGT 25.6-29.7°C	Tympanic pre and post exercise (ECT)	45 km cycling TT	Body mass loss (kg). Converted to WBSR (mL·min^{-1})
Fujii et al. (2021)	Single-blind, randomised, placebo-controlled, cross-over	Healthy, physically active, non-heat acclimatised males (*n* = 12). Age 23 ± 2 years	Caffeine 5 mg·kg^{-1} (70 min pre-exercise)	37°C 50% RH WBGT 31°C	Oesophageal continuously (T_{core} rate of rise °C/hr)	45 min cycling @ 55% $\dot{V}O_{2peak}$	Sweat loss (L). Converted to WBSR (mL·min^{-1})

Ganio et al. (2011)	Double-blind, randomised, placebo-controlled, cross-over	Healthy, trained, non-heat acclimated male cyclists (*n* = 11). Age 25 ± 6 years	Caffeine 3 mg·kg^{-1} (60 min pre-exercise)	33°C 41% RH WBGT 26.1°C	Rectal every 15 min (ECT)	90 min cycling @ 65% thermoneutral $\dot{V}O_{2max}$ followed by 15 min TT	Sweat rate (L·h^{-1}). Converted to WBSR (mL·min^{-1})
Gordon et al. (1982)	Double-blind, independent design	Healthy, fit males (*n* = 10; 5 *vs* 5). Age 19.4 ± 1.5 years	Caffeine 5 mg·kg^{-1} (60 min pre-exercise)	26.7°C average, ranged from 24.5-28.9°C 41-54% RH WBGT 18.9-24.1°C	Rectal pre- and post-exercise (ECT)	120 min running	Sweat loss (kg). Converted to WBSR (mL·min^{-1})
Hanson et al. (2019)[2] A and B	Single-blind, randomised, placebo-controlled, cross-over	Trained male (*n* = 6) and female (*n* = 4) endurance runners (*n* = 10). Age 26 ± 9 years	Caffeine 3 mg·kg^{-1} (60 min pre-exercise) A Caffeine 6 mg·kg^{-1} (60 min pre-exercise) B	30.6°C 50% RH WBGT 25.2°C	Gastrointestinal every 1 km (ECT)	10 km running TT	No sweating response data reported
Hunt et al. (2021)[3] A and B	Double-blind, randomised, counter-balanced, placebo-controlled, cross-over	Healthy, non-heat acclimated, caffeine habituated males (*n* = 10) and females (*n* = 4; *n* = 14; A) and caffeine non-habituated males (*n* = 8) and females (*n* = 6; *n* = 14; B). Age 27 ± 5 *vs* 23 ± 3 years	Caffeine 5 mg·kg^{-1} (60 min pre-exercise)	30.6°C 31% RH WBGT 22.6°C	Oesophageal every 5 s (T_{core} rate of rise °C/hr)	60 min cycling @ 7 W·kg^{-1} $\dot{H}_{prod}$	WBSL (kg). Converted to WBSR (mL·min^{-1}). LSR at the back and arm (mg·min·cm^{-1}; ventilated capsule technique)

Kim & Lee (2013)[1]	Randomised, cross-over	Healthy males (*n* = 9). Age 24.1 ± 3.5 years	Caffeine 3 mg·kg^{-1} (60 min pre-trial)	25°C 60% RH WBGT 21.3°C 42°C bath	No T_{core} data reported	30 min water immersion up to umbilical line	WBSL volume (mL). Converted to WBSR (mL·min^{-1})
Millard-Stafford et al. (2007)	Double-blind, randomised, repeated-measures, placebo-controlled, cross-over	Healthy, highly trained male cyclists (*n* = 16). Age 27.5 ± 7 years	Caffeine 1.2 mg·kg^{-1} (0 min pre-exercise) and 3.5 mg·kg^{-1} (at 60 min)	28°C 60% RH WBGT 24°C	Rectal every 5 min (ECT)	120 min cycling @ alternating 15 mins of 60 and 70% $\dot{V}O_{2max}$ followed by 15 min TT	Sweat rate (mL·h^{-1}). Converted to WBSR (mL·min^{-1})
Nakamura et al. (2020)	Double-blind, randomised, placebo-controlled, cross-over	Trained male footballers (*n* = 8). Age 19.9 ± 0.3 years.	Caffeine 3 mg·kg^{-1} (60 min pre-trial)	31.7°C 63.5% RH WBGT 27.9°C	Rectal every 30 s (ECT)	2 x 43 min bouts consisting of 21 cycling intermittent sprints	Sweat volume (L). Converted to WBSR (mL·min^{-1})
Ping et al. (2010)[2]	Double-blind, randomised, placebo-controlled, cross-over	Recreational, heat acclimated male runners (*n* = 9). Age 25.4 ± 6.9 years	Caffeine 5 mg·kg^{-1} (60 min pre-exercise)	31°C 70% RH WBGT 27.9°C	Rectal every 10 min (Peak CT)	Treadmill running @ 70% $\dot{V}O_{2max}$	No sweating response data reported
Pitchford et al. (2014)	Double-blind, randomised, counter-balanced, placebo-controlled, cross-over	Highly trained, non-heat acclimated male cyclists (*n* = 9). Age range 22-42 years	Caffeine 3 mg·kg^{-1} (90 min pre-exercise)	35°C 25% RH WBGT 25.2°C	Gastrointestinal continuously (ECT)	Total work cycling TT	Body weight loss (kg). Converted to WBSR (mL·min^{-1})
Roelands et al. (2011)	Double-blind, randomised,	Healthy, trained, non-heat	Caffeine	30°C	Rectal every 5 min (ECT)	60 min cycling @ 55% W_{max}	Sweat rate (mL·min^{-1}).

	placebo-controlled, cross-over	acclimated males (*n* = 8). Age 23 ± 5 years	6 mg·kg^{-1} (60 min pre-exercise)	50-60% RH WBGT 24.6-25.9°C		followed by total work TT	
Roti et al. (2006) A and B	Double-blind, randomised, independent design	Healthy, active males (*n* = 59; 20 *vs* 20 *vs* 19). Age 21.6 ± 3.1 years	Caffeine 3 mg·kg^{-1} (6 x 3 mg·kg·d^{-1}; no timing mentioned) A Caffeine 6 mg·kg^{-1} (6 x 6 mg·kg·d^{-1}; no timing mentioned) B	37.7°C 56.3% RH WBGT 32.4°C	Rectal every 15 min (ECT)	90 min treadmill walking @ 1.56 m·s^{-1} with a 5% incline	Sweat rate (L·h^{-1}). Converted to WBSR (mL·min^{-1})
Stebbins et al. (2001)[2]	Double-blind, randomised, placebo-controlled, cross-over	Healthy, active males (*n* = 11). Age range 18-40 years	Caffeine 6 mg·kg^{-1} (0 min pre-trial and 45 min pre-exercise)	38°C 40% RH WBGT 30.2°C	Rectal periodically (Mean T_{core})	40 min resting followed by 35 min cycling @ 50% $\dot{V}O_{2max}$	No sweating response data reported
Suvi et al. (2017)[1]	Double-blind, randomised, placebo-controlled, cross-over	Healthy, physically active, non-heat acclimated males (*n* = 13) and females (*n* = 10; *n* = 23). Age 24.9 ± 4.1 *vs* 22.5 ± 2 years	Caffeine 6 mg·kg^{-1} (4 mg·kg^{-1} 60 min and 2 mg·kg^{-1} 0 min pre-exercise)	42°C 20% RH WBGT 29.5°C	Rectal every 1 min (ECT)	50 min treadmill walking @ 60% thermoneutral $\dot{V}O_{2peak}$ followed by TTE	Sweat production (mL·min^{-1})
Kern et al. (2001)	Double-blind, randomised, independent design	Healthy moderately-highly active males (*n* = 20; 10 *vs* 10). *Age* 22.3 ± 3.6 years.	Creatine 335 g (5 x 21 g·d^{-1} followed by 23 x 10 g·d^{-1})	37°C 25% RH WBGT 26.7°C	Rectal every 15 min (ECT)	60 min cycling @ 60% $\dot{V}O_{2max}$	Body weight loss (%)
Kilduff et al. (2004)	Double-blind, randomised,	Endurance-trained, non-heat acclimated males	Creatine 159.6 g (7 x 22.8 g·d^{-1})	30.3°C 70% RH	Rectal every 5 min (ECT)	Cycling @ incremental work rate at 60-90 rpm	Sweat rate (mL·min^{-1})

	independent design	(*n* = 21; 11 *vs* 10). Age 27 ± 5 *vs* 27 ± 4 years		WBGT 27.2°C			
Branch et al. (2007)	Double-blind, randomised, counter-balanced, placebo-controlled, cross-over	Healthy, competitive male cyclists and triathletes (*n* = 7). Age 38 ± 7 years	Creatine 100 g (5 x 20 g·d^{-1})	38°C 35% RH WBGT 29.3°C	Tympanic every 10 min (ECT)	60 min cycling @ 50% $\dot{V}O_{2max}$	Body mass loss (%)
Mendel et al. (2005)	Double-blind, independent design	Healthy, recreationally active, non-heat acclimated males (*n* = 15) and female (*n* = 1; *n* = 16 8 *vs* 8). Age 26 ± 3.6 *vs* 26 ± 1.9 years	Creatine 100 g (5 x 20 g·d^{-1})	39°C 26% RH WBGT 28.5°C	Rectal every 10 min (ECT)	40 min cycling @ 55% $\dot{V}O_{2max}$	Weight loss (kg). Converted to WBSR (mL·min^{-1})
Rosene et al. (2015)[2]	Double-blind, randomised, cross-over	Regularly exercising males (*n* = 14). Age 21.1 ± 1.4 years.	Creatine 0.3 g·kg·d^{-1} (3 x 0.3 g·kg·d^{-1})	32.6°C 18.5% RH WBGT 22.1°C	Rectal every 5 min (ECT)	60 min treadmill running @ 60-65% $\dot{V}O_{2max}$	No sweating response data reported
Vogel et al. (2000)[1]	Randomised, independent design	Healthy, recreationally active, non-heat acclimated males (*n* = 16; 7 *vs* 9). Age 22 ± 1 years	Creatine 100 g (5 x 20 g·d^{-1})	32°C 50% RH WBGT 26.5°C	T_{core} not measured	2 x 75 min exercise (4 x 10 min cycling bouts @ 30% initial sprint resistance at 60 rpm)	Body weight change (%)
Volek et al. (2001)	Double-blind, randomised, independent design	Healthy males (*n* = 20; 10 *vs* 10). Age 23 ± 1 years	Creatine 0.3 g·kg^{-1})7 x 0.3 g·kg^{-1})	37°C 80% RH WBGT 34.8°C	Rectal every 5 min (ECT)	15 min cycling @ 70% $\dot{V}O_{2peak}$, followed by 15 min @ 60% $\dot{V}O_{2peak}$,	Sweat rate (mL·min^{-1})

						followed by 3 x 10 s maximal sprints)	
Watson et al. (2006)	Double-blind, randomised, placebo-controlled, cross-over	Healthy, physically active, non-heat acclimated males (*n* = 12). Age 22 ± 1 years	Creatine 194 g (9 x 21.6 g·d^{-1})	33.5°C 41% RH WBGT 26.4°C	Rectal every 20 min (ECT)	80 min treadmill exercise (4 x 20 min sequences of 4 min resting, alternating 3 min walking, 1 min run x 3 and 4 min walk)	Sweat loss (kg). Converted to WBSR (mL·min^{-1})
Weiss & Powers (2006)	Double-blind, randomised, counter-balanced, independent design	Healthy, aerobically trained males (*n* = 24; 12 *vs* 12). Age 22.9 ± 3.0 years	Creatine 125 g (5 x 25 g·d^{-1})	37°C %RH – not mentioned	Gastrointestinal every 10 min (ECT)	60 min cycling @ 70% age predicted maximum HR	Sweat loss (kg). Converted to WBSR (mL·min^{-1})
Wright et al. (2007)	Single-blind, intervention	Physically active, heat acclimatised males (*n* = 10). Age 25.7 ± 4.9 years	Creatine 120 g (6 x 20 g·d^{-1})	35°C 60% RH WBGT 30.4°C	Rectal continuously (ECT)	6 x 10 s maximal cycling sprints	Sweat loss (kg). Converted to WBSR (mL·min^{-1})
Kent et al. (2018)[2]	Double-blind, repeated-measures, counter-balanced, placebo-controlled, cross-over	Endurance-trained male cyclists (*n* = 12). Age 26.6 ± 4.4 years	Nitrate (NO_3^-) 13 mmol (2 x 6.5 mmol·d^{-1} and 13 mmol 120 min pre-exercise)	35°C 48% RH WBGT 28.9°C	Gastrointestinal every 20% work rate (ECT)	Total work cycling TT	No sweat response data extractable
Kent et al. (2018)	Double-blind, counter-balanced, placebo-	Endurance trained male cyclists (*n* = 12). Age 27 ± 6 years.	Nitrate (NO_3^-) 13 mmol (2 x 6.5 mmol·d^{-1} and 13 mmol 120 min pre-trial)	33.3°C 48.8% RH WBGT 27.5°C	Gastrointestinal every 5 min (ECT)	60 min cycling @ 60% $\dot{V}O_{2peak}$	Sweat loss (L). Converted to WBSR (mL·min^{-1})

	controlled, cross-over						
Amano et al. (2018)[2,3]	Double-blind, randomised, placebo-controlled, cross-over	Healthy, active males (*n* = 5) and females (*n* = 3; *n* = 8). Age 24 ± 4 years	Nitrate (NO_3^-) 8 mmol (2 x 8 mmol·d^{-1} and 8 mmol 120 min pre-exercise)	30°C 50% RH WBGT 24.6°C	Oesophageal continuously (ECT)	30 min cycling @ 55% $\dot{V}O_{2max}$	LSR on the left ventral forearm and chest (mg·min·cm^{-1}; ventilated capsule technique)
Cramer et al. (2020)	Intervention	Healthy males (*n* = 3) and females (*n* = 6; *n* = 9). Age 67 ± 5 years	Nitrate (NO_3^-) 16.8 mmol (6 x 16.8 mmol·d^{-1} and 16.8 mmol 120 min pre-trial)	42.5°C 34.2% RH WBGT 33°C	Gastrointestinal continuously (ECT)	120 min resting in a reclining chair	WBSL (kg). Converted to WBSR (mL·min^{-1})
Fowler et al. (2020)[3]	Double-blind, randomised, placebo-controlled, cross-over	Healthy, physically inactive, non-heat acclimated males (*n* = 11). Age 25 ± 5 years	Nitrate (NO_3^-) 9.2 mmol (5 x 9.2 mmol·d^{-1})	35°C 28% RH WBGT 25.7°C	Rectal every 1 min (ECT)	Cycling @ thermoneutral gas exchange threshold at 70 rpm	Body mass change (%). LSR on the chest, forearm, thigh and calf (nL·min^{-1}; ventilated capsule technique)
Kuennen et al. (2015)	Double-blind, randomised, counter-balanced, placebo-controlled, cross-over	Healthy, recreationally active males (*n* = 9). Age 24 ± 1 years	Nitrate (NO_3^-) 4.2 mmol (6 x 8.4 mmol·d^{-1}, with 4.2 mmol 2.5 h pre-trial)	41.2°C 15% RH WBGT 27.8°C	Rectal every 5 s (ECT)	45 min treadmill walking @ 4.83 km·h^{-1} with a 1.5% incline	Sweat rate (mL·min^{-1})
McQuillan et al. (2018)	Double-blind, randomised, placebo-	Healthy, well-trained endurance male cyclists (*n* =	Nitrate (NO_3^-) 8 mmol (2 x 8 mmol·d^{-1} and 8	35°C 60% RH	Rectal continuously (ECT)	20 min cycling @ 40-60% PPO, followed by 4 km TT	Sweat loss (L·h^{-1}). Converted to

	controlled, cross-over	8). Age 25 ± 8 years	mmol 90 min pre-exercise)	WBGT 30.4°C			WBSR ($mL \cdot min^{-1}$)
Smith et al. (2019)[2]	Double-blind, randomised, counter-balanced, placebo-controlled, cross-over	Recreationally trained males (*n* = 12), Age 22 ± 4 years	Nitrate (NO_3^-) 6.2 mmol (180 min pre-exercise)	30°C 70% RH WBGT 26.9°C	Tympanic post IST (ECT)	20 x 6s cycling sprints (114s active recovery)	No sweating response data reported
Nava et al. (2019)[2]	Double-blind, randomised, placebo-controlled, cross-over	Healthy, physically active, non-heat acclimated males (*n* = 7) and females (*n* = 4; *n* = 11). Age 28.3 ± 6.8 years	L-glutamine 0.15 $g \cdot kg^{-1}$ (60 min pre-exercise)	38°C 35% RH WBGT 29.3°C	Rectal (Peak T_{core})	87 min simulated fire-fighting exercise	No sweating response data reported
Ogden et al. (2022)	Double-blind, randomised, counter-balanced, placebo-controlled, cross-over	Healthy, recreationally active, non-heat acclimated males (*n* = 10). Age 29 ± 7 years	L-glutamine 0.3 $g \cdot kg^{-1}$ FFM (60 min pre-exercise)	40.3°C 38% RH WBGT 31.8°C	Rectal every 10 min (ECT)	30 min treadmill running @ normothermic anaerobic LT	Sweat rate ($L \cdot h^{-1}$). Converted to WBSR ($mL \cdot min^{-1}$)
Ogden et al. (2022)	Double-blind, randomised, counter-balanced, placebo-controlled, cross-over	Healthy, recreationally active, non-heat acclimated males (*n* = 12). Age 32 ± 6 years	L-glutamine 0.3 $g \cdot kg^{-1}$ FFM (60 min pre-exercise)	35.3°C 30.5% RH WBGT 26.3°C	Rectal every 20 min (ECT)	2 x 40 min bouts treadmill walking @ 6 $km \cdot h^{-1}$ with a 7% incline	WBSL ($L \cdot h^{-1}$). Converted to WBSR ($mL \cdot min^{-1}$)
Osborne et al. (2019)	Double-blind, randomised, placebo-controlled, cross-over	Healthy, trained, male cyclists (*n* = 12). Age 32 ± 6 years	L-glutamine 0.9 $g \cdot kg^{-1}$ FFM (60 min pre-exercise)	35.1°C 51% RH WBGT 29.4°C	Rectal every 2 s (Mean T_{core})	20 km cycling TT	Body mass loss (kg). Converted to WBSR ($mL \cdot min^{-1}$)

Pugh et al. (2017)[2] A, B and C	Double-blind, randomised, placebo-controlled, cross-over	Healthy, recreationally active males (*n* = 10). Age 24 ± 4 years	L-glutamine 0.25 g·kg^{-1} FFM (120 min pre-exercise) A L-glutamine 0.5 g·kg^{-1} FFM (120 min pre-exercise) B L-glutamine 0.9 g·kg^{-1} FFM (120 min pre-exercise) C	30°C 40-45% RH WBGT 23.3-24°C	Rectal continuously (Mean T_{core})	60 min treadmill running @ 70% $\dot{V}O_{2max}$	No sweating response data reported
Zheng et al. (2018)	Double-blind, randomised, placebo-controlled, cross-over	Healthy, untrained males (*n* = 13). Age 20.2 ± 1.1 years	L-glutamine 0.6 g·kg^{-1} (30 min pre-exercise)	38°C 60% RH WBGT 33.2°C	Gastrointestinal continuously (ECT)	Treadmill running @ 40% $\dot{V}O_{2max}$	Body weight loss (kg). Converted to WBSR (mL·min^{-1})
Zuhl et al. (2014)[2]	Double-blind, counter-balanced, placebo-controlled, cross-over	Healthy, endurance trained males (*n* = 8). Age 25 ± 4 years	L-glutamine 0.9 g·kg^{-1} FFM (7 x 0.9 g·kg·ffm·d^{-1}; 120 min pre-exercise)	30°C 12-20% RH WBGT 19.2-20.4°C	Rectal (no timing mentioned; ECT)	60 min treadmill running @ 65-70% $\dot{V}O_{2max}$	No sweating response data reported
Zuhl et al. (2015)[2]	Double-blind, placebo-controlled, cross-over	Healthy, endurance trained males (*n* = 2) and females (*n* = 5; *n* = 7). Age 26 ± 4 years	L-glutamine 0.9 g·kg^{-1} FFM (120 min pre-exercise)	30°C 12-20 % RH WBGT 19.2-20.4°C	Rectal (no timing mentioned; ECT)	60 min treadmill running @ 70% $\dot{V}O_{2max}$	No sweating response data reported
Lee & Shin (2014)[2,3]	Placebo-controlled, cross-over	Healthy males (*n* = 19). Age 23.7 ± 2.3 years	Oligonol 200 mg (7 x 200 mg)	26°C 60% RH	Tympanic (no timing	30 min half body water immersion	LSR on the chest, back, abdomen and

				WBGT 22.2°C 42°C bath	mentioned; ECT)		thigh (mg·min·cm^{-1}; ventilated capsule technique)
Lee et al. (2015)[1]	Placebo-controlled, cross-over	Healthy males (*n* = 19). Age 23.7 ± 2.3 years	Oligonol 200 mg (7 x 200 mg)	26°C 60% RH WBGT 22.2°C 42°C bath	Tympanic (no timing mentioned; ECT)	30 min half body water immersion	WBSL volume (mL·30 min). Converted to WBSR (mL·min^{-1})
Shin et al. (2011)[2]	Randomised, placebo-controlled, cross-over	Healthy males (*n* = 13). Age 21.8 ± 2.3 years.	Oligonol 100 mg (30 min pre-trial)	26°C 60% RH WBGT 22.2°C 43°C bath	Tympanic (no timing mentioned; ECT)	30 min lower leg water immersion	No sweating response data reported
Shin et al. (2013)[2]	Double-blind, randomised, placebo-controlled, cross-over	Healthy males (*n* = 17). Age 21.6 ± 2.1 years	Oligonol 100 mg (60 min pre-trial)	26°C 60% RH WBGT 22.2°C 42°C bath	Tympanic (no timing mentioned; ECT)	30 min half body water immersion	No sweating response data reported
Trinity et al. (2014)[2]	Double-blind, randomised, placebo-controlled, cross-over	Healthy, well-trained male cyclists (*n* = 12). Age 26.8 ± 5 years	Polyphenols 3600-ppm (7 x 3600-ppm·d^{-1})	31.5°C 55% RH WBGT 26.7°C	Rectal continuously (ECT)	20 min cycling @ 40, 50, 60 and 70% followed by 30 min cycling @ 5% above L_T, followed by 10 min TT @ 90% $\dot{V}O_{2max}$	Body mass loss (kg). Converted to WBSR (mL·min^{-1})

Szymanski et al. (2018)	Double-blind, counter-balanced, placebo-controlled, cross-over	Healthy, recreationally active, non-heat acclimated males (*n* = 6) and females (*n* = 2; *n* = 8). Age 19 ± 1 years	Curcumin 300 mg (3 x 500 mg·d^{-1}, with 300 mg 60 min pre-exercise)	37°C 25% RH WBGT 26.7°C	Oesophageal every 5 min (ECT)	60 min treadmill running @ 65% $\dot{V}O_{2max}$	Sweat rate (L·h^{-1}). Converted to WBSR (mL·min^{-1})
Cheuvront et al. (2004)	Placebo-controlled, cross-over	Healthy, physically active, moderately fit, heat acclimated males (*n* = 7). Age 21 ± 2 years	BCAAs 14 g (0 min pre- and during exercise)	40°C 20% RH WBGT 28°C	Rectal every 10 min (ECT)	60 min cycling @ 50% $\dot{V}O_{2peak}$ followed by 30 min TT	Fluid loss (%)
Macedo et al. (2019)[2]	Double-blind, randomised, placebo-controlled, cross-over	Physically active males (*n* = 9). Age 25.4 ± 1.2 years	BCAAs 30 mg·kg^{-1} (120, 60 and 0 min pre-exercise and every 30 min during exercise)	35°C 60% RH WBGT 30.4°C	Rectal every 30 s (ECT)	Cycling @ 40% peak power at 50 rpm	No sweating response data reported
Mittleman et al. (1998)	Double-blind, placebo-controlled, cross-over	Healthy, moderately-trained males (*n* = 7) and females (*n* = 6; *n* = 13). Age 24 ± 2.9 *vs* 25.6 ± 7 years	BCAAs Females (9.4 g) and males (15.8 g; 5 mL·kg^{-1} of 5.88 g·L^{-1} (every 60 min at rest and 30 min during exercise)	34.4°C 39% RH WBGT 27°C	Oesophageal every 5 min (ECT)	Cycling @ 40% $\dot{V}O_{2peak}$	Sweat loss (L). Converted to WBSR (mL·min^{-1})
Watson et al. (2004)[2]	Double-blind, randomised, placebo-controlled, cross-over	Healthy, endurance exercising, non-heat acclimated males (*n* = 8). Age 28.5 ± 8.2 years	BCAAs 4 x 250 ml at 12 g·L^{-1} (30 min intervals pre-exercise and 150 ml every 15 min during exercise)	30°C 38% RH WBGT 23.1°C	Rectal every 10 min (ECT)	Cycling @ 50% $\dot{V}O_{2peak}$	No sweating response data reported
Coull et al. (2016)	Double-blind, counter-	Recreationally active, non-heat	Tyrosine	40°C	Rectal every 5 min (ECT)	60 min treadmill walk followed by	Sweat loss (L). Converted to

	balanced, placebo-controlled, cross-over	acclimated males (*n* = 8). Age 23 ± 1 years	150 mg·kg^{-1} (60 min pre-exercise)	30% RH WBGT 30.1°C		2.4 km TT wearing a 25 kg backpack	WBSR (mL·min^{-1})
Kishore et al. (2021)[2]	Double-blind, randomised, placebo-controlled, cross-over	Healthy males (*n* = 10). Age range 20-30 years	Tyrosine 6.5 g (90 min pre-trial)	45°C 30% RH WBGT 34.1°C	Oral temperature (End)	90 min resting	No sweating response data reported
Tumilty et al. (2011)	Double-blind, randomised, placebo-controlled, cross-over	Healthy, endurance exercising, non-heat acclimated males (*n* = 8). Age 32 ± 11 years	Tyrosine 150 mg·kg^{-1} (60 pre-exercise)	30°C 60% RH WBGT 25.9°C	Rectal every 10 min (ECT)	Cycling @ 68% $\dot{V}O_{2peak}$	Body mass loss (kg). Converted to WBSR (mL·min^{-1})
Tumilty et al. (2014)	Double-blind, randomised, placebo-controlled, cross-over	Endurance exercising, non-heat acclimated males (*n* = 7). Age 20 (range 26) years	Tyrosine 150 mg·kg^{-1} (60 pre-exercise)	30°C 60% RH WBGT 25.9°C	Rectal every 5 min (ECT)	60 min cycling @ 57% $\dot{V}O_{2peak}$ followed by total work TT	Body mass loss rate (kg·h^{-1}). Converted to WBSR (mL·min^{-1})
Tumilty et al. (2020) A, B and C	Double-blind, randomised, placebo-controlled, cross-over	Healthy, recreationally active, non-heat acclimated males (*n* = 8). Age 23 ± 4 years	Tyrosine 150 mg·kg^{-1} (timing not mentioned) A Tyrosine 300 mg·kg^{-1} (timing not mentioned) B Tyrosine 400 mg·kg^{-1} (timing not mentioned) C	30°C 60% RH WBGT 25.9°C	Rectal continuously (ECT)	60 min cycling @ 10% delta of the $\dot{V}O_2$ at GET plus 10% of the difference between GET and $\dot{V}O_{2peak}$, followed by a individualised work target TT	Body mass change (%)

Watson et al. (2012)	Randomised, counter-balanced, placebo-controlled, cross-over	Physically active, trained, non-heat acclimated males (*n* = 8). Age 23 ± 3 years	Tyrosine 150 mg·kg^{-1} (120 min, 60 min and during)	30°C 50% RH WBGT 24.6°C	Rectal every 5 min (ECT)	Cycling @ 70% $\dot{V}O_{2peak}$	Sweat rate (mL·min^{-1})
Page et al. (2019)[3]	Double-blind, randomised, placebo-controlled, cross-over	Healthy, non-heat acclimated males (*n* = 11). Age 23 ± 2 years.	Taurine 50 mg·kg^{-1} (120 min pre-exercise)	35°C 40% RH WBGT 27.6°C	Rectal every 1 min (ECT)	Cycling @ thermoneutral ventilatory threshold at 80 rpm	Body mass change (g). Converted to WBSR (mL·min^{-1}). LSR on the chest, upper-arm thigh and calf (nL·min^{-1}; ventilated capsule technique)
Armstrong et al. (2008)[2]	Double-blind, randomised, placebo-controlled, cross-over	Healthy, well-trained male runners (*n* = 10). Age 20 ± 2 years.	Betaine 5 g (45 min pre-exercise)	31.1°C 34.7% RH WBGT 23.6°C	Rectal periodically (ECT)	75 min treadmill running @ 65% $\dot{V}O_{2max}$ followed by TTE @ 84% $\dot{V}O_{2max}$	Whole-body sweat rate (L·h^{-1}). No sweating data extractable
Willingham et al. (2023)	Double-blind, randomised, placebo-controlled, cross-over	Healthy, recreationally active males (*n* = 11). Age 29.1 ± 5.2 years	Betaine 50 mg·kg^{-1} (7 x 50 mg·kg·d^{-1})	40°C 60% RH WBGT 35.1°C	Gastrointestinal every 15 min (ECT)	60 min resting	Sweat rate (L·h^{-1}). Converted to WBSR (mL·min^{-1})
Tyler et al. (2016)	Double-blind, placebo-controlled, cross-over	Healthy, recreationally active, non-heat acclimated males (*n* = 8). Age 27 ± 6 years	L-arginine 10 g (30 min pre-trial)	35°C 50% RH WBGT 29.1°C	Rectal every 5 min (ECT)	90 min resting, followed by 30 min cycling @ 60% W_{max}, followed by 30 min resting	Body mass loss (kg). Converted to WBSR (mL·min^{-1})

Carrillo et al. (2008)[2]	Double-blind, randomised, independent design	Healthy, aerobically fit males (*n* = 8) and females (*n* = 4; *n* = 12; 6 *vs* 6). Age 23.4 ± 4.6 years.	Ascorbic acid (vitamin C) 1500 mg (9 x 1500 mg·d^{-1} and 8 mmol 120 min pre-exercise)	34.8°C 13% RH WBGT 22.8°C	Rectal pre and post exercise (ECT)	180 min cycling @ 55% $\dot{V}O_{2max}$	No sweating response data extractable
Kotze et al. (1977) A and B	Placebo-controlled, independent design	Non-heat acclimated, males (*n* = 13; 4 *vs* 5 *vs* 4). Age 23 ± 3 *vs* 24 ± 2 *vs* 20 ± 2.9 years	Ascorbic acid (vitamin C) 250 mg (180-240 min pre-exercise) A Ascorbic acid (vitamin C) 500 mg (180-240 min pre-exercise) B	33.9°C %RH (did not mention)	Rectal every 60 min (ECT)	240 min block stepping @ 35 W workload	Sweat output (kg). Converted to WBSR (mL·min^{-1})
Keong et al. (2006)[2]	Double-blind, randomised, placebo-controlled, cross-over	Recreational, heat acclimated male athletes (*n* = 18). Age 24.9 ± 1.4 years	Vitamin E No dose stated (6 weeks)	31°C 70% RH WBGT 27.9°C	Rectal every 10 min (ECT)	Treadmill running @ 70% $\dot{V}O_{2max}$	No sweating response data reported
Muhamad et al. (2010)	Double-blind, randomised, placebo-controlled, cross-over	Healthy, male recreational athletes (*n* = 12). Age 23.3 ± 3.7 years	E. longifolia Jack 150 mg (7 x 150 mg·d^{-1} and 150 mg 60 min pre-exercise)	31°C 70% RH WBGT 27.9°C	Tympanic every 10 min (ECT)	60 min treadmill running @ 60% $\dot{V}O_{2max}$ followed by 20 min TT	Sweat rate (L·h^{-1}). Converted to SR (mL·min^{-1})
Easton et al. (2007) A, B[2] and C	Double-blind, randomised, intervention	Healthy, endurance trained males (*n* = 23; 12 [creatine and glycerol; creatine) *vs* 11 [glycerol]). Age 31 ± 7 years	Glycerol 1 g·kg^{-1} (6 x 1 g·d^{-1} and 1 g 5 h pre-trial) A Creatine 120 g (6 x 20 g·d^{-1} and 10 g 5 h pre-trial) B	30°C 70% RH WBGT 26.9°C	Rectal every 5 min (ECT)	40 min cycling @ 63% WR_{max} followed by 16.1 km TT	Sweat rate (L·h^{-1}). Converted to WBSR (mL·min^{-1})

			Creatine 120 g (6 x 20 g·d^{-1} and 10 g 5 h pre-trial) AND Glycerol 1 g·kg^{-1} (6 x 1 g·d^{-1} and 1 g 5 h pre-trial) C				
Gill et al. (2016)	Blinded, randomised, counter-balanced, placebo-controlled, cross-over	Healthy, endurance trained, non-heat acclimated male runners (*n* = 8). Age 26 ± 6 years	Probiotic L.casei 100 billion (7 x 10^{11}·d^{-1})	34°C 32% RH WBGT 25.5°C	Rectal every 10 min (Mean T_{core})	120 min treadmill running @ 60% $\dot{V}O_{2max}$	Body mass loss (%)
Shing et al. (2014)	Double-blind, randomised, counter-balanced, placebo-controlled, cross-over	Healthy, trained, non-heat acclimated, male runners (*n* = 10). Age 27 ± 2 years	Probiotics 28 capsules (28 x 1 capsule·kg·d^{-1} = 45 billion colony forming units)	35°C 40% RH WBGT 27.6°C	Gastrointestinal every 1 min (Maximum T_{core})	Running @ 80% V_T	Body mass loss (kg). Converted to WBSR (mL·min^{-1})
Hiles et al. (2020)	Double-blind, randomised, placebo-controlled, cross-over	Healthy, recreationally active males (*n* = 12) and females (*n* = 6; *n* = 18). Age 27 ± 6 years	Blackcurrant extract 600 mg (7 x 600 mg·d^{-1})	34.1°C 40.8% RH WBGT 27°C	Rectal every 10 min (Mean T_{core})	60 min treadmill running @ 65% $\dot{V}O_{2max}$ with a 1% incline	Whole-body sweat rate (L·h^{-1}). Converted to WBSR (mL·min^{-1})
Lee et al. (2022)	Double-blind, randomised, placebo-controlled, cross-over	Healthy, recreationally active males (*n* = 12). Age 28 ± 6 years	Blackcurrant extract 600 mg (7 x 600 mg·d^{-1})	34.1°C 40.8% RH WBGT 27°C	Rectal every 10 min (ECT)	60 min treadmill running @ 70% $\dot{V}O_{2max}$ with a 1% incline	Sweat rate (L·h^{-1}). Converted to WBSR (mL·min^{-1})

March et al. (2019)[2]	Double-blind, randomised, placebo-controlled, cross-over	Healthy, regularly exercising males (*n* = 12). Age 26 ± 6 years	Bovine colostrum 20 g (14 x 20 g·d^{-1})	30°C 60% RH WBGT 25.9°C	Rectal every 10 min (ECT)	60 min treadmill running @ 70% $\dot{V}O_{2max}$ with a 1% incline	No sweating response data reported
McKenna et al. (2020)	Double-blind, randomised, counter-balanced, placebo-controlled, cross-over	Healthy, active males (*n* = 10). Age 20 ± 2 years	Bovine colostrum 20 g (14 x 20 g·d^{-1})	40°C 50% RH WBGT 33.5°C	Gastrointestinal every 5 min (ECT)	~46 min treadmill running @ 95% V_T	Sweat rate (mL·min^{-1})
Morrison et al. (2014)[2] A and B	Double-blind, randomised, placebo-controlled, cross-over	Healthy, trained (*n* = 7; A) and untrained (*n* = 8; B; *n* = 15) males. Age 23 ± 4 *vs* 21 ± 2 years	Bovine colostrum 1.7 g·kg (7 x 1.7 g·kg·d^{-1})	30°C 50% RH WBGT 24.6°C	Oesophageal every 1 min (ECT)	15 min cycling @ 50% HRR, followed by 30 min treadmill running @ 80% HRR, followed by 30 min TT, followed by 15 min cycling @ 50% HRR	No sweating response data reported
Miyazawa et al. (2012)[3]	Double-blind, randomised, placebo-controlled, cross-over	Healthy, moderately active males (*n* = 8). Age 23.5 ± 3.6 years	GABA 1 g (0 min pre-trial)	33°C 50% RH WBGT 27.3°C	Oesophageal (no timing mentioned; ECT)	30 min resting	Sweat loss (g). Converted to WBSR (mL·min^{-1}). LSR on the chest (mg·min·cm^{-1}; ventilated capsule technique)
Taylor et al. (2016)[2]	Double-blind, randomised, cross-over	Males (*n* = 6). Age 22.0 ± 1.3 years	Effective microorganism X 70 ml (7 x 70 ml·d^{-1})	34.7°C 51.7% RH	Rectal every 5 min (ECT)	20 x 10 s IST @ maximal running velocity, with 80 s	No sweating response data reported

				WBGT 29°C		active recover @ 35% $\dot{V}O_{2max}$	
Vaher et al. (2015)	Double-blind, randomised, placebo-controlled, cross-over	Healthy, endurance trained, non-heat acclimated males (*n* = 16). Age 25.8 ± 4.4 years	Sodium citrate 500 mg·kg^{-1} (120 min pre-exercise)	32°C 50% RH WBGT 26.5°C	Rectal every 1 min (ECT)	5 km treadmill running TT	Body mass loss (kg). Converted to WBSR (mL·min^{-1})
Suvi et al. (2018)	Double-blind, randomised, placebo-controlled, cross-over	Healthy, endurance trained males (*n* =20). Age 30.8 ± 5.4 years	Sodium citrate 200 mg·kg^{-1} (3 x 200 mg·kg·d^{-1}, consumed the day before, the evening before and 120 min pre-exercise)	32°C 46% RH WBGT 25.9°C	Rectal every 1 min (ECT)	40 km cycling TT	Sweat production (L·h^{-1}). Converted to WBSR (mL·min^{-1})
Zabriskie et al. (2020)	Double-blind, randomised, counter-balanced, placebo-controlled. cross-over	Healthy, recreationally active males (*n* = 16) and females (*n* = 15; *n* = 31). Age 29.6 ± 6.7 *vs* 30.1 ± 8.9 years	Beta-glucan 250 mg (11 x 250 mg·d^{-1})	37.2°C 45.2% RH WBGT 30.3°C	Gastrointestinal every 10 min (ECT)	60 min treadmill walking @ 55% $\dot{V}O_{2peak}$	Body weight loss (kg). Converted to WBSR (mL·min^{-1})
Gagnon et al. (2018)[3]	Intervention	Healthy males (*n* = 3) and females (*n* = 6; *n* = 9). Age 68 ± 3 years	Folic acid 5 mg (42 x 5 mg·d^{-1})	42°C 30-70% RH WBGT 31.7-38.3°C	Oesophageal continuously (ECT)	100 min resting	WBSL (kg). Converted to WBSR (mL·min^{-1}). LSR on the forearm (mg·min·cm^{-1}; ventilated capsule technique)

Ping et al. (2011)[2]	Double-blind, randomised, placebo-controlled, cross-over	Recreational, heat acclimated male runners (*n* = 9). Age 25.4 ± 6.9 years	Ginseng 200 mg (60 min pre-exercise)	31°C 70% RH WBGT 27.9°C	Rectal every 10 min (ECT)	Treadmill running @ 70% $\dot{V}O_{2max}$	No sweating response data reported
Vogel et al. (2023)[2]	Double-blind, randomised, placebo-controlled, cross-over	Healthy, non-heat acclimated, endurance trained male (*n* = 8) and female (*n* = 6) runners (*n* = 14). Age 31 ± 6 years	Menthol gel 16 g (0.5% menthol; 5 min pre-exercise and 50 and 40 min mid trial.	33°C 49% RH WBGT 27.2°C	Gastrointestinal every 10 min (ECT)	40 min treadmill running @ 60% HR_{max} at $\dot{V}O_{2max}$, followed by 20 min TT with a 1% incline	Body mass loss (kg). Converted to WBSR ($mL \cdot min^{-1}$).
Riera et al. (2014)[2]	Randomised, placebo-controlled, cross-over	Healthy, heat acclimated, trained male cyclists and triathletes (*n* = 12). Age 42 ± 13 years	Menthol aroma 190 ml (0.5 g/L; 0.01% menthol) 15 and 0 min pre-trial and every 5 km	30.7°C 78% RH WBGT 28.6°C	Gastrointestinal pre, post and every 5 km (ECT)	20 km cycling TT	No sweating response data reported
Bandyopadhyay et al. (2011)[2]	Double-blind, randomised, placebo-controlled, cross-over	Recreational, heat acclimated male runners (*n* = 9). Age 25.4 ± 6.9 years	Caffeine 5 $mg \cdot kg^{-1}$ AND Ginseng 200 mg (60 min pre-exercise)	31°C 70% RH WBGT 27.9°C	Rectal every 10 min (ECT)	Treadmill running @ 70% $\dot{V}O_{2max}$	No sweating response data reported
Nishimura et al. (2019)[2,3]	Placebo-controlled, cross-over	Healthy males (*n* =8). Age 26 ± 8 years	Catechin 121 mg/100ml (4 $ml \cdot kg^{-1}$ 3 x (0, 30 and 60 min during)	35°C 75% RH WBGT 32.3°C 40°C bath	Tympanic (no timing mentioned; ECT)	90 min lower leg water immersion	LSR on the upper arm ($mg \cdot min \cdot cm^{-1}$; ventilated capsule technique)
Pokora et al. (2019)[2]	Double-blind, randomised, placebo-controlled, cross-over	Healthy males (*n* = 12) and females (*n* = 13; *n* = 25). Age 23 ± 1.3 years	Thermo Speed Extreme (green tea extract 5.1 $mg \cdot kg^{-1}$, synephrine 0.3	26°C 56% RH WBGT 21.8°C	Tympanic periodically (ECT)	6 h resting	No sweating response data reported

			mg·kg^{-1} and caffeine 3 mg·kg^{-1}; 0 min pre-trial)				
Snipe et al. (2017)	Randomised, cross-over	Healthy non heat acclimatised, endurance trained male (*n* = 6) and female (*n* = 5) runners (*n* = 11). Age 31 ± 5 years	Whey protein hydrolysate 15 g (0 min pre-exercise and every 20 min during)	35.5°C 27% RH WBGT 25.9°C	Rectal every 10 mins (ECT)	120 min treadmill running @ 60% $\dot{V}O_{2max}$	Body mass loss (%)
Polyviou et al. (2012) A and B	Double-blind, randomised, intervention	Healthy, endurance trained males (*n* = 18; 9 *vs* 9). Age 31.5 ± 9 years	Creatine 20 g, Glycerol 2 mg AND Glucose 150 g (7 x creatine 20 g·d^{-1}, glycerol 2 mg·kg·d^{-1} and glucose 150 g·d^{-1}) A Creatine 20 g, Glycerol 2 mg, Glucose 100 g AND Alpha lipoic acid 1000 mg (7 x creatine 20 g·d^{-1}, glycerol 2 mg·kg·d^{-1}, glucose 100 g·d^{-1} and alpha lipoic acid 1000 mg) B	30°C 70% RH WBGT 26.9°C	No method mentioned, measured every 5 min (ECT)	40 min cycling @ pre-determined work rate, followed by 16.1 km TT	Sweat loss (ml). Converted to WBSR (mL·min^{-1})

Anderson & Hickey (1994)[2]	Double-blind, counter-balanced, placebo-controlled, cross-over	Moderately trained males (*n* = 8). Age 24 ± 3 years	Caffeine 5 mg·kg^{-1} (30 min pre-exercise)	28°C 50% RH WBGT 22.9°C	Rectal every 10 min (ECT)	60 min cycling @ 50% $\dot{V}O_{2max}$	No sweating response data reported
MacNaughton et al. (1990)[2]	Double-blind, counter-balanced, placebo-controlled, cross-over	Healthy males (*n* = 6). Age 22 range 19-25 years	Caffeine 5 mg·kg^{-1} (no timing mentioned)	28°C 42% RH WBGT 22°C	Rectal (no timing mentioned; ECT)	120 min resting	No sweating response data reported
Kazman et al. (2020)	Double-blind, randomised, placebo-controlled, cross-over	Healthy males and females (*n* = 32). Age 27 ± 8 years	Caffeine 7.5 mg·kg^{-1} (60 min pre-exercise)	40°C 40% RH WBGT 31.9°C	Rectal (no timing mentioned; ECT)	60 min treadmill walking @ 5 km·h^{-1} with 2% incline, followed by a 5 min stepping test (24 steps·min^{-1}) and 15 deep knee bends	Sweat rate (L·h^{-1}). Converted to WBSR (mL·min^{-1})
Beis et al. (2011)	Intervention	Healthy males (*n* = 14). Age 27 ± 8 years	Creatine 10 g, Glycerol 1 g·kg^{-1} AND Glucose 75 g (6 x creatine 10 g·d^{-1}, glycerol 1 g·kg^{-1} and glucose 75 g·d^{-1} and 5 h pre-exercise)	35.1°C 69.4% RH WBGT 31.8°C	Gastrointestinal every 5 min (ECT)	30 min treadmill running @ 60% $\dot{V}O_{2max}$, with a 1% incline	Sweat loss (L). Converted to WBSR (mL·min^{-1})
Miyazawa et al. (2009)	Randomised, placebo-controlled, cross-over	Healthy, exercise trained males (*n* = 8). Age 22.8 ± 3.7 years	GABA 1 g (20 min pre-trial)	35°C 50% RH	Oesophageal (no timing mentioned; ECT)	30 min semirecumbent cycling @ 65% $\dot{V}O_{2peak}$ at 60 rpm	Sweat loss (g). Converted to WBSR (mL·min^{-1})

				WBGT 29.1°C			
Kavouras et al. (2006)	Double-blind, randomised, placebo-controlled, cross-over	Healthy, endurance trained male cyclists (*n* = 8). Age 24 ± 3 years	Glycerol 1 g·kg^{-1} (30 min pre-exercise)	36.8°C 48.1% RH WBGT 30.5°C	Rectal every 4 min (ECT)	Cycling @ 74% $\dot{V}O_{2peak}$	Sweating (ml). Converted to WBSR (mL·min^{-1})
Anderson et al. (2001)[2]	Double-blind, randomised, placebo-controlled, cross-over	Endurance trained males (*n* = 6). Age 23.3 ± 6.6 years	Glycerol 1 g·kg^{-1} (120 min pre-exercise)	35°C 30% RH WBGT 26°C	Rectal every 15 min (ECT)	90 min cycling @ 98% L_T, followed by 15 min TT	No sweating response data reported
Desroches et al. (2023)	Randomised, placebo-controlled, cross-over	Healthy, recreationally active males (*n* = 9) and females (*n* = 1; *n* = 10). Age 24 ± 4 years	Glycerol 1.4 g·kg^{-1} FFM (120, 100, 80 and 60 min pre-exercise)	30°C 50% RH WBGT 24.6°C	Gastrointestinal (no timing mentioned)	5 km treadmill running TT	Sweat loss (mL).
Wingo et al. (2004)	Double-blind, randomised, repeated-measures, placebo-controlled, cross-over	Heat acclimatised male mountain bikers (*n* = 12). Age 24.5 ± 3.8 years	Glycerol 1 g·kg^{-1} (no timing mentioned)	WBGT 28.1°C	Rectal every 16 km (ECT)	48 km mountain-bicycle race	Sweat rate (L·h^{-1}). Converted to WBSR (mL·min^{-1})
Coutts et al. (2002)[1]	Randomised, placebo-controlled, cross-over	Well-trained heat acclimatised male (*n* = 7) and female (*n* = 3) triathletes (*n* = 10). Age 33.3 ± 7.3 years	Glycerol 1.2 g·kg^{-1} (130 min pre-exercise)	30.1-45.6°C 46.3-51.7% RH WBGT 23.9-32.9°C	No T_{core} data reported	Olympic distance triathlon	Sweat loss (%)
Katagiri et al. (2021)[3]	Blinded, placebo-	Healthy males (*n* = 11). Age 23 ± 4 years	Sodium bicarbonate	35°C 40% RH	Oesophageal every 1 s (ECT)	60 min cycling @ 50% $\dot{V}O_{2peak}$	Body weight loss (kg). Converted to

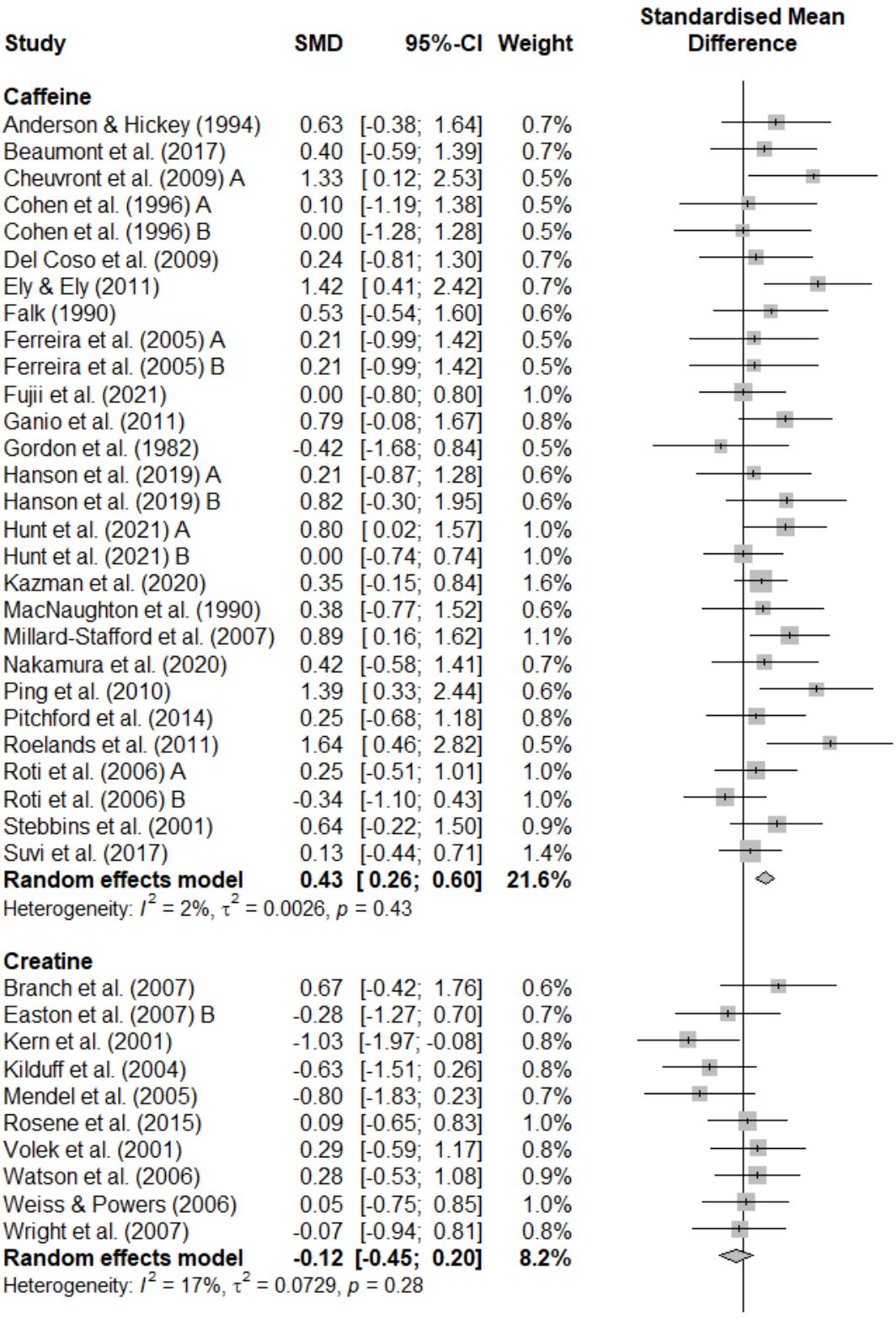
	controlled, cross-over		300 mg·kg^{-1} (90 min pre-exercise)	WBGT 27.6°C			WBSR (mL·min^{-1}).
Katagiri et al. (2023)[3]	Counter-balanced, placebo-controlled, cross-over	Healthy males (*n* = 13). Age 24 ± 2 years	Sodium bicarbonate 300 mg·kg^{-1} (95 min pre-exercise)	35°C 50% RH WBGT 29.1°C	Oesophageal every 1 s (ECT)	60 min cycling @ 50% $\dot{V}O_{2peak}$	Body weight loss (%). Converted to WBSR (mL·min^{-1}). LSR on the left forearm and chest (mg·min·cm^{-1}; ventilated capsule technique)
Dini et al. (2007)[1] A and B	Randomised, independent design	High-level oarsmen (*n* = 14; 5 *vs* 5 *vs* 4). Age 26 ± 5 years	Glycerol 1 g·kg^{-1} (180 min pre-exercise) A Glycerol 1 g·kg^{-1} (1 g·kg^{-1} 180 min pre-exercise and 23 and 61 min during) B	36°C 30% RH WBGT 26.8°C	Rectal continuously (ECT)	89 min rowing	Fluid loss (mL). Converted to WBSR (mL·min^{-1})
Hitchins et al. (1999)	Double-blind, counter-balanced, placebo-controlled, cross-over	Trained, non-heat acclimated male cyclists (*n* = 8). Age 27 ± 4.2 years	Glycerol 1 g·kg^{-1} (150 min pre-exercise)	33.2°C 57.8% RH WBGT 28.6°C	Rectal every 5 min (ECT)	60 min cycling (30 min @ fixed power output, followed by 30 min @ self-paced power output)	Sweat loss (%)
Hillman et al. (2013)	Randomised, placebo-controlled, cross-over	Healthy, non-heat acclimated trained male cyclists (*n* = 7). Age 28 ± 8 years	Glycerol 1.2 g·kg^{-1} (120 min pre-exercise)	35°C 40% RH WBGT 27.6°C	Rectal every 5 min (ECT)	90 min cycling TT	Body mass change (%)

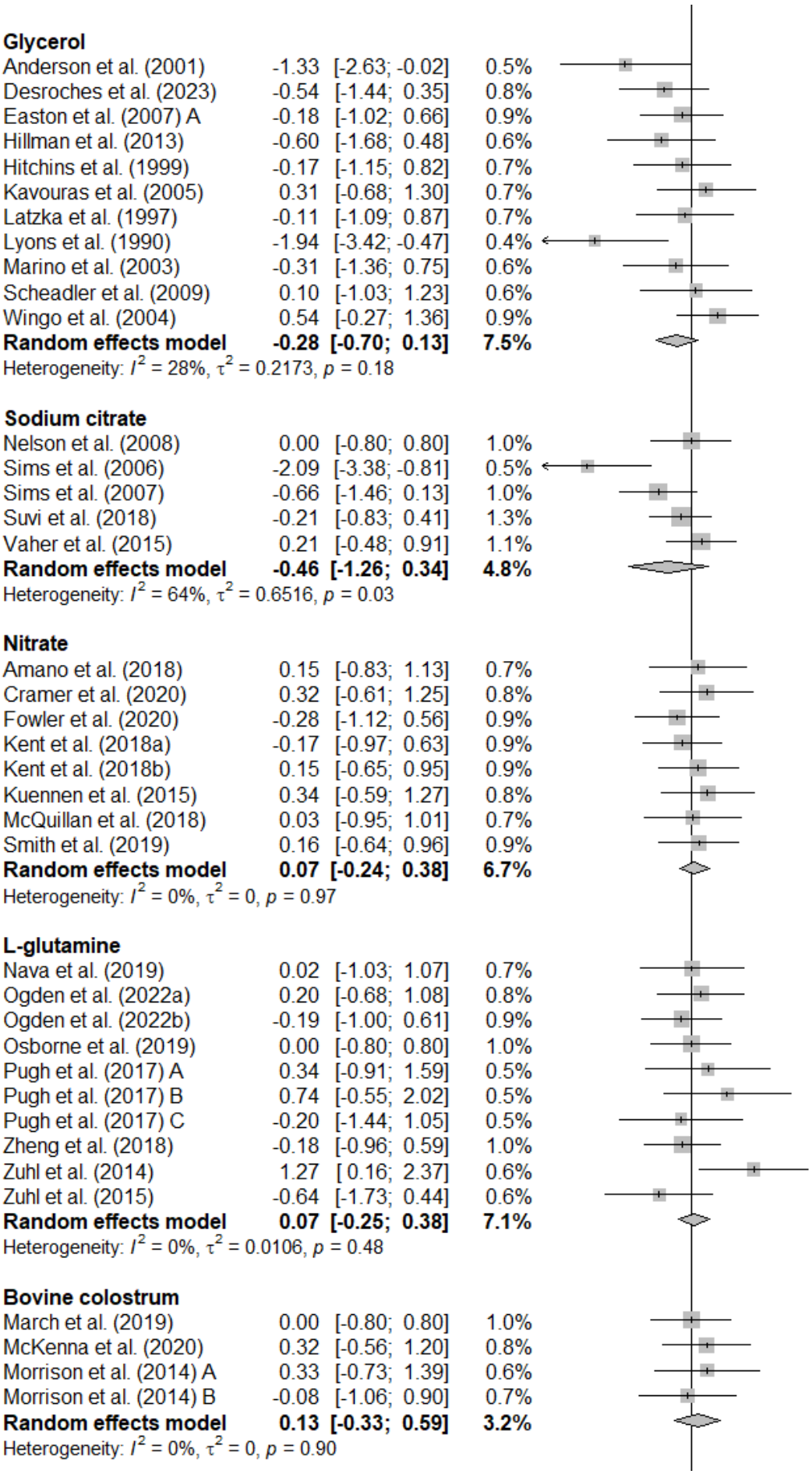
Marino et al. (2003)	Double-blind, randomised, placebo-controlled, cross-over	Healthy, moderately-to-well trained males (*n* = 6) and females (*n* = 1; *n* = 7). Age 21.2 ± 2.4 years	Glycerol 1.2 g·kg^{-1} (150 min pre-exercise)	34.5°C 63.4% RH WBGT 30.5°C	Rectal every 5 min (ECT)	60 min cycling TT	Sweat rate (L·h^{-1}). Converted to WBSR (mL·min^{-1})
Sims et al. (2007)	Double-blind, randomised, placebo-controlled, cross-over	Healthy, non-heat acclimatised, endurance trained males (*n* = 8). Age 36 ± 11 years	Sodium citrate 7.72 g AND Sodium chloride 4.5 g (45 min pre-exercise)	32°C 50% RH WBGT 26.5°C	Rectal every 30 s (ECT)	Treadmill running @ 70% $\dot{V}O_{2max}$	Sweat loss rate (L·h^{-1}). Converted to WBSR (mL·min^{-1})
Sims et al. (2007)	Double-blind, randomised, counter-balanced, placebo-controlled, cross-over	Healthy, non-heat acclimatised, endurance trained females (*n* = 13). Age 26 ± 6 years	Sodium citrate 7.72 g AND Sodium chloride 4.5 g (20 min pre-exercise)	32°C 50% RH WBGT 26.5°C	Rectal every 1 min (ECT)	Treadmill running @ 70% $\dot{V}O_{2max}$	Sweat loss rate (L·h^{-1}). Converted to WBSR (mL·min^{-1})
Latzka et al. (1997)[3]	Double-blind, randomised, placebo-controlled, cross-over	Healthy, heat acclimated males (*n* = 8). Age 23 ± 6 years	Glycerol 1.2 g·kg^{-1} (no timing mentioned)	34.9°C % RH (not reported) WBGT 30.3°C	Rectal and Oesophageal (no timing mentioned)	120 min treadmill exercise @ 45% $\dot{V}O_{2max}$	Whole-body sweating rate (g·m^{-2}·h^{-1}). Local sweating rate of the upper arm (mg·min·cm^{-1}; ventilated capsule technique)
Lyons et al. (1990)	Randomised, placebo-controlled, cross-over	Healthy, heat acclimatised males (*n* = 4) and females (*n* = 2; *n* = 6). Age 26.2 ± 3.7 years	Glycerol 1 g·kg^{-1} (150 min pre-exercise)	42°C 25% RH WBGT 30.6°C	Rectal every 15 min (ECT)	90 min treadmill exercise @ 60% $\dot{V}O_{2max}$	Sweat output (mL). Converted to WBSR (mL·min^{-1}).

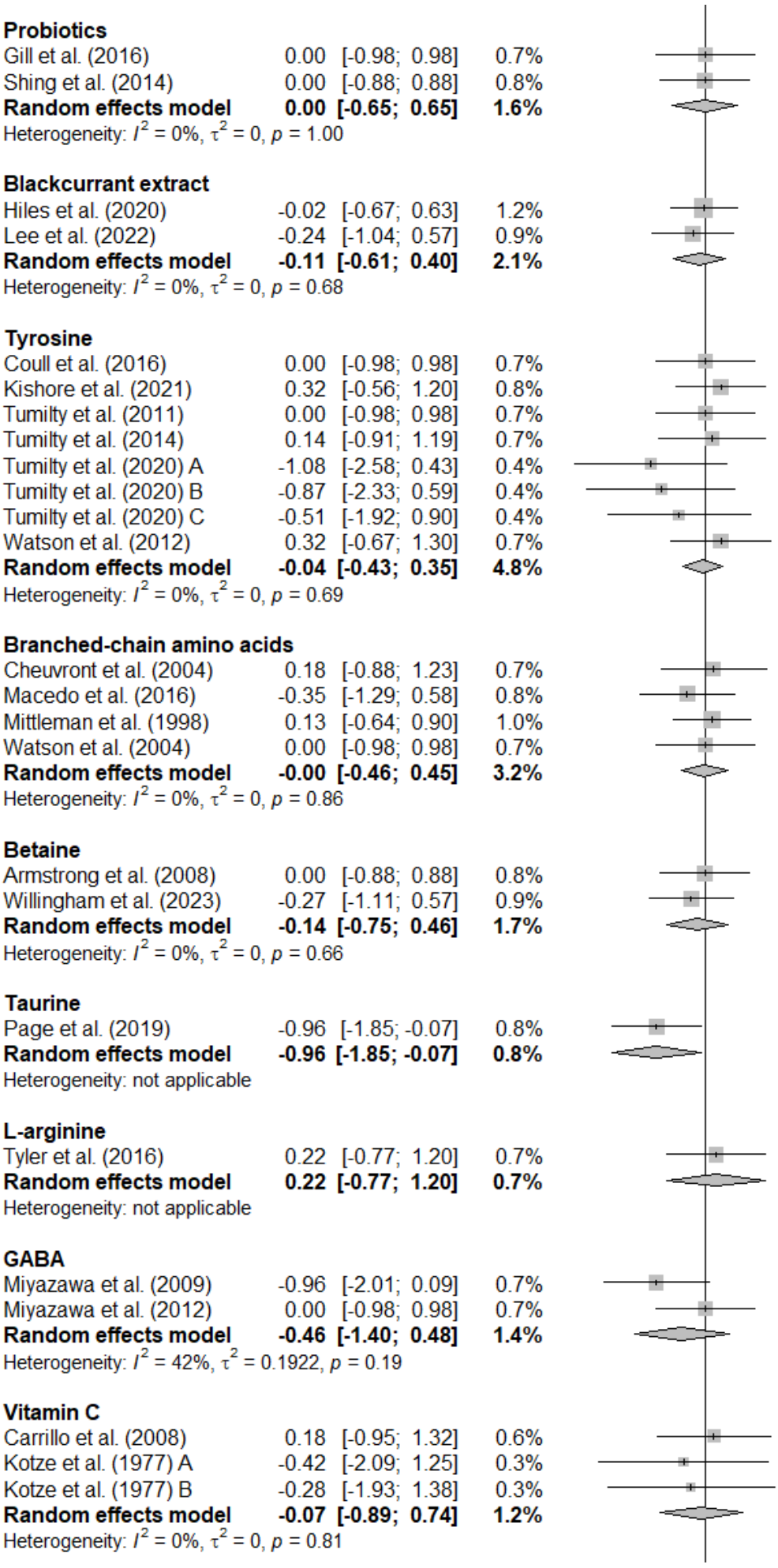
McCullagh et al. (2013)[1]	Double-blind, randomised, placebo-controlled, cross-over	Healthy, well-trained males (*n* = 5) and females (*n* = 1; *n* = 6). No age provided	Glycerol 1.2 g·kg^{-1} (120 min pre-exercise)	30°C % RH (not reported) WBGT 30.3°C	No T_{core} data reported	150 min exercise (10 km treadmill running and 40 km cycling @ a set load [~177 W] followed by 5 km treadmill running TT)	Body weight loss (kg). Converted to WBSR (mL·min^{-1}).
Scheadler (2009)	Double-blind, randomised, placebo-controlled, cross-over	Healthy, non-heat acclimatised, endurance trained males (*n* = 6). Age 27.8 ± 6 years	Glycerol 1.2 g·kg^{-1} (140 min pre-exercise)	30°C 50% RH WBGT 24.6°C	Gastrointestinal (no timing mentioned; ECT)	Set distance treadmill running @ ~83% $\dot{V}O_{2peak}$	Sweat rate (L·h^{-1}). Converted to WBSR (mL·min^{-1}).
Nelson et al. (2008)	Blinded, randomised, placebo-controlled, cross-over	Healthy, non-heat acclimatised, moderately trained males (*n* = 12). Age 24.3 ± 4.2 years	Sodium citrate 0.2 g·kg^{-1} (100 min pre-exercise)	30.9°C 63.8% RH WBGT 27.2°C	Rectal every 5 min (ECT)	62 min cycling @ 15% below V_T (~60% $\dot{V}O_{2peak}$)	Sweat loss (L). Converted to WBSR (mL·min^{-1}).
Kuennen et al. (2011)	Double-blind, counter-balanced, placebo-controlled, cross-over	Healthy, non-heat acclimated, physically active males (*n* = 8). Age 28 ± 4.8 years	Quercetin 2000 mg·d^{-1} (with breakfast)	46.6°C 21% RH WBGT 33.3°C	Rectal continuously (ECT)	45 min treadmill running @ 50% $\dot{V}O_{2max}$	WBSR (mL·min^{-1}).
Klarod et al. (2015)	Randomised, placebo-controlled, cross-over	Healthy, regularly active males (*n* = 7). Age 2.7 ± 2.6 years	α-KG 4.8 g AND 5-HMF 60 mg (48 h pre-trial)	33°C 40% RH WBGT 26°C	Tympanic pre and post exercise (ECT)	Treadmill running @ 1 km·h·min^{-1} increases	No sweating response data reported

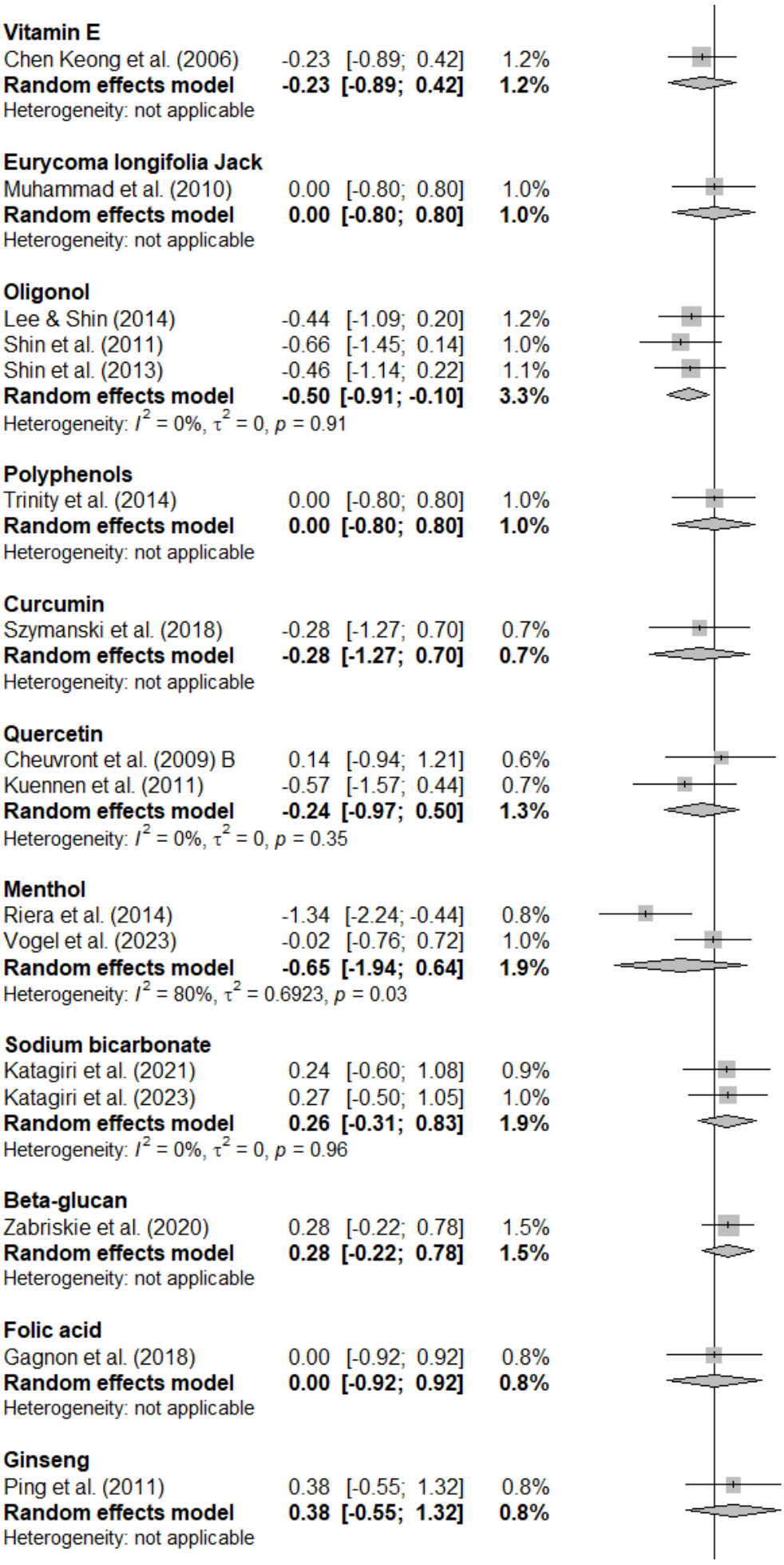
TT time-trial, *TTE* time-to-exhaustion, *IST* intermittent-sprint-test, *PPO* peak power output, *ECT* end core temperature, T_{core} core temperature, *RH* relative humidity, *WBGT* wet-bulb globe temperature, $\dot{V}O_{2max}$ maximal oxygen uptake, $\dot{V}O_{2peak}$ peak oxygen uptake, W_{max} watt maximum, WR_{max} work rate maximum, $\dot{H}_{prod}$ heat production, *PPO* peak power output, *HR* heart rate, L_T lactate threshold, *GET* gas exchange threshold, V_T ventilatory threshold, *rpm* revolution per minute, *WBSR* whole-body sweat rate, *WBSL* whole-body sweat loss, *LSR* local sweat rate, *BCAAs* branched-chain amino acids, *GABA* gamma-aminobutyric

acid, α-KG alpha-ketoglutaric acid, 5-HMF 5-hydroxymethylfurfural, [1] not included in end core temperature analysis, [2] not included in the sweating response analysis, [3] included in local sweat rate analysis. The table is a reflection of the studies, as reported by the authors of the original articles.


Study
SMD
95%-CI
Weight
Standardised Mean Difference
Caffeine
Anderson & Hickey (1994) 0.63 [-0.38; 1.64] 0.7%
Beaumont et al. (2017) 0.40 [-0.59; 1.39] 0.7%
Cheuvront et al. (2009) A 1.33 [0.12; 2.53] 0.5%
Cohen et al. (1996) A 0.10 [-1.19; 1.38] 0.5%
Cohen et al. (1996) B 0.00 [-1.28; 1.28] 0.5%
Del Coso et al. (2009) 0.24 [-0.81; 1.30] 0.7%
Ely & Ely (2011) 1.42 [0.41; 2.42] 0.7%
Falk (1990) 0.53 [-0.54; 1.60] 0.6%
Ferreira et al. (2005) A 0.21 [-0.99; 1.42] 0.5%
Ferreira et al. (2005) B 0.21 [-0.99; 1.42] 0.5%
Fujii et al. (2021) 0.00 [-0.80; 0.80] 1.0%
Ganio et al. (2011) 0.79 [-0.08; 1.67] 0.8%
Gordon et al. (1982) -0.42 [-1.68; 0.84] 0.5%
Hanson et al. (2019) A 0.21 [-0.87; 1.28] 0.6%
Hanson et al. (2019) B 0.82 [-0.30; 1.95] 0.6%
Hunt et al. (2021) A 0.80 [0.02; 1.57] 1.0%
Hunt et al. (2021) B 0.00 [-0.74; 0.74] 1.0%
Kazman et al. (2020) 0.35 [-0.15; 0.84] 1.6%
MacNaughton et al. (1990) 0.38 [-0.77; 1.52] 0.6%
Millard-Stafford et al. (2007) 0.89 [0.16; 1.62] 1.1%
Nakamura et al. (2020) 0.42 [-0.58; 1.41] 0.7%
Ping et al. (2010) 1.39 [0.33; 2.44] 0.6%
Pitchford et al. (2014) 0.25 [-0.68; 1.18] 0.8%
Roelands et al. (2011) 1.64 [0.46; 2.82] 0.5%
Roti et al. (2006) A 0.25 [-0.51; 1.01] 1.0%
Roti et al. (2006) B -0.34 [-1.10; 0.43] 1.0%
Stebbins et al. (2001) 0.64 [-0.22; 1.50] 0.9%
Suvi et al. (2017) 0.13 [-0.44; 0.71] 1.4%
Random effects model 0.43 [0.26; 0.60] 21.6%
Heterogeneity: I^2 = 2%, τ^2 = 0.0026, p = 0.43
Creatine
Branch et al. (2007) 0.67 [-0.42; 1.76] 0.6%
Easton et al. (2007) B -0.28 [-1.27; 0.70] 0.7%
Kern et al. (2001) -1.03 [-1.97; -0.08] 0.8%
Kilduff et al. (2004) -0.63 [-1.51; 0.26] 0.8%
Mendel et al. (2005) -0.80 [-1.83; 0.23] 0.7%
Rosene et al. (2015) 0.09 [-0.65; 0.83] 1.0%
Volek et al. (2001) 0.29 [-0.59; 1.17] 0.8%
Watson et al. (2006) 0.28 [-0.53; 1.08] 0.9%
Weiss & Powers (2006) 0.05 [-0.75; 0.85] 1.0%
Wright et al. (2007) -0.07 [-0.94; 0.81] 0.8%
Random effects model -0.12 [-0.45; 0.20] 8.2%
Heterogeneity: I^2 = 17%, τ^2 = 0.0729, p = 0.28


Glycerol
Anderson et al. (2001) -1.33 [-2.63; -0.02] 0.5%
Desroches et al. (2023) -0.54 [-1.44; 0.35] 0.8%
Easton et al. (2007) A -0.18 [-1.02; 0.66] 0.9%
Hillman et al. (2013) -0.60 [-1.68; 0.48] 0.6%
Hitchins et al. (1999) -0.17 [-1.15; 0.82] 0.7%
Kavouras et al. (2005) 0.31 [-0.68; 1.30] 0.7%
Latzka et al. (1997) -0.11 [-1.09; 0.87] 0.7%
Lyons et al. (1990) -1.94 [-3.42; -0.47] 0.4%
Marino et al. (2003) -0.31 [-1.36; 0.75] 0.6%
Scheadler et al. (2009) 0.10 [-1.03; 1.23] 0.6%
Wingo et al. (2004) 0.54 [-0.27; 1.36] 0.9%
Random effects model -0.28 [-0.70; 0.13] 7.5%
Heterogeneity: I^2 = 28%, τ^2 = 0.2173, p = 0.18
Sodium citrate
Nelson et al. (2008) 0.00 [-0.80; 0.80] 1.0%
Sims et al. (2006) -2.09 [-3.38; -0.81] 0.5%
Sims et al. (2007) -0.66 [-1.46; 0.13] 1.0%
Suvi et al. (2018) -0.21 [-0.83; 0.41] 1.3%
Vaher et al. (2015) 0.21 [-0.48; 0.91] 1.1%
Random effects model -0.46 [-1.26; 0.34] 4.8%
Heterogeneity: I^2 = 64%, τ^2 = 0.6516, p = 0.03
Nitrate
Amano et al. (2018) 0.15 [-0.83; 1.13] 0.7%
Cramer et al. (2020) 0.32 [-0.61; 1.25] 0.8%
Fowler et al. (2020) -0.28 [-1.12; 0.56] 0.9%
Kent et al. (2018a) -0.17 [-0.97; 0.63] 0.9%
Kent et al. (2018b) 0.15 [-0.65; 0.95] 0.9%
Kuennen et al. (2015) 0.34 [-0.59; 1.27] 0.8%
McQuillan et al. (2018) 0.03 [-0.95; 1.01] 0.7%
Smith et al. (2019) 0.16 [-0.64; 0.96] 0.9%
Random effects model 0.07 [-0.24; 0.38] 6.7%
Heterogeneity: I^2 = 0%, τ^2 = 0, p = 0.97
L-glutamine
Nava et al. (2019) 0.02 [-1.03; 1.07] 0.7%
Ogden et al. (2022a) 0.20 [-0.68; 1.08] 0.8%
Ogden et al. (2022b) -0.19 [-1.00; 0.61] 0.9%
Osborne et al. (2019) 0.00 [-0.80; 0.80] 1.0%
Pugh et al. (2017) A 0.34 [-0.91; 1.59] 0.5%
Pugh et al. (2017) B 0.74 [-0.55; 2.02] 0.5%
Pugh et al. (2017) C -0.20 [-1.44; 1.05] 0.5%
Zheng et al. (2018) -0.18 [-0.96; 0.59] 1.0%
Zuhl et al. (2014) 1.27 [0.16; 2.37] 0.6%
Zuhl et al. (2015) -0.64 [-1.73; 0.44] 0.6%
Random effects model 0.07 [-0.25; 0.38] 7.1%
Heterogeneity: I^2 = 0%, τ^2 = 0.0106, p = 0.48
Bovine colostrum
March et al. (2019) 0.00 [-0.80; 0.80] 1.0%
McKenna et al. (2020) 0.32 [-0.56; 1.20] 0.8%
Morrison et al. (2014) A 0.33 [-0.73; 1.39] 0.6%
Morrison et al. (2014) B -0.08 [-1.06; 0.90] 0.7%
Random effects model 0.13 [-0.33; 0.59] 3.2%
Heterogeneity: I^2 = 0%, τ^2 = 0, p = 0.90


Probiotics
Gill et al. (2016) 0.00 [-0.98; 0.98] 0.7%
Shing et al. (2014) 0.00 [-0.88; 0.88] 0.8%
Random effects model 0.00 [-0.65; 0.65] 1.6%
Heterogeneity: I^2 = 0%, τ^2 = 0, p = 1.00
Blackcurrant extract
Hiles et al. (2020) -0.02 [-0.67; 0.63] 1.2%
Lee et al. (2022) -0.24 [-1.04; 0.57] 0.9%
Random effects model -0.11 [-0.61; 0.40] 2.1%
Heterogeneity: I^2 = 0%, τ^2 = 0, p = 0.68
Tyrosine
Coull et al. (2016) 0.00 [-0.98; 0.98] 0.7%
Kishore et al. (2021) 0.32 [-0.56; 1.20] 0.8%
Tumilty et al. (2011) 0.00 [-0.98; 0.98] 0.7%
Tumilty et al. (2014) 0.14 [-0.91; 1.19] 0.7%
Tumilty et al. (2020) A -1.08 [-2.58; 0.43] 0.4%
Tumilty et al. (2020) B -0.87 [-2.33; 0.59] 0.4%
Tumilty et al. (2020) C -0.51 [-1.92; 0.90] 0.4%
Watson et al. (2012) 0.32 [-0.67; 1.30] 0.7%
Random effects model -0.04 [-0.43; 0.35] 4.8%
Heterogeneity: I^2 = 0%, τ^2 = 0, p = 0.69
Branched-chain amino acids
Cheuvront et al. (2004) 0.18 [-0.88; 1.23] 0.7%
Macedo et al. (2016) -0.35 [-1.29; 0.58] 0.8%
Mittleman et al. (1998) 0.13 [-0.64; 0.90] 1.0%
Watson et al. (2004) 0.00 [-0.98; 0.98] 0.7%
Random effects model -0.00 [-0.46; 0.45] 3.2%
Heterogeneity: I^2 = 0%, τ^2 = 0, p = 0.86
Betaine
Armstrong et al. (2008) 0.00 [-0.88; 0.88] 0.8%
Willingham et al. (2023) -0.27 [-1.11; 0.57] 0.9%
Random effects model -0.14 [-0.75; 0.46] 1.7%
Heterogeneity: I^2 = 0%, τ^2 = 0, p = 0.66
Taurine
Page et al. (2019) -0.96 [-1.85; -0.07] 0.8%
Random effects model -0.96 [-1.85; -0.07] 0.8%
Heterogeneity: not applicable
L-arginine
Tyler et al. (2016) 0.22 [-0.77; 1.20] 0.7%
Random effects model 0.22 [-0.77; 1.20] 0.7%
Heterogeneity: not applicable
GABA
Miyazawa et al. (2009) -0.96 [-2.01; 0.09] 0.7%
Miyazawa et al. (2012) 0.00 [-0.98; 0.98] 0.7%
Random effects model -0.46 [-1.40; 0.48] 1.4%
Heterogeneity: I^2 = 42%, τ^2 = 0.1922, p = 0.19
Vitamin C
Carrillo et al. (2008) 0.18 [-0.95; 1.32] 0.6%
Kotze et al. (1977) A -0.42 [-2.09; 1.25] 0.3%
Kotze et al. (1977) B -0.28 [-1.93; 1.38] 0.3%
Random effects model -0.07 [-0.89; 0.74] 1.2%
Heterogeneity: I^2 = 0%, τ^2 = 0, p = 0.81


Vitamin E
Chen Keong et al. (2006) -0.23 [-0.89; 0.42] 1.2%
Random effects model **-0.23 [-0.89; 0.42] 1.2%**
Heterogeneity: not applicable
Eurycoma longifolia Jack
Muhammad et al. (2010) 0.00 [-0.80; 0.80] 1.0%
Random effects model **0.00 [-0.80; 0.80] 1.0%**
Heterogeneity: not applicable
Oligonol
Lee & Shin (2014) -0.44 [-1.09; 0.20] 1.2%
Shin et al. (2011) -0.66 [-1.45; 0.14] 1.0%
Shin et al. (2013) -0.46 [-1.14; 0.22] 1.1%
Random effects model **-0.50 [-0.91; -0.10] 3.3%**
Heterogeneity: I^2 = 0%, τ^2 = 0, p = 0.91
Polyphenols
Trinity et al. (2014) 0.00 [-0.80; 0.80] 1.0%
Random effects model **0.00 [-0.80; 0.80] 1.0%**
Heterogeneity: not applicable
Curcumin
Szymanski et al. (2018) -0.28 [-1.27; 0.70] 0.7%
Random effects model **-0.28 [-1.27; 0.70] 0.7%**
Heterogeneity: not applicable
Quercetin
Cheuvront et al. (2009) B 0.14 [-0.94; 1.21] 0.6%
Kuennen et al. (2011) -0.57 [-1.57; 0.44] 0.7%
Random effects model **-0.24 [-0.97; 0.50] 1.3%**
Heterogeneity: I^2 = 0%, τ^2 = 0, p = 0.35
Menthol
Riera et al. (2014) -1.34 [-2.24; -0.44] 0.8%
Vogel et al. (2023) -0.02 [-0.76; 0.72] 1.0%
Random effects model **-0.65 [-1.94; 0.64] 1.9%**
Heterogeneity: I^2 = 80%, τ^2 = 0.6923, p = 0.03
Sodium bicarbonate
Katagiri et al. (2021) 0.24 [-0.60; 1.08] 0.9%
Katagiri et al. (2023) 0.27 [-0.50; 1.05] 1.0%
Random effects model **0.26 [-0.31; 0.83] 1.9%**
Heterogeneity: I^2 = 0%, τ^2 = 0, p = 0.96
Beta-glucan
Zabriskie et al. (2020) 0.28 [-0.22; 0.78] 1.5%
Random effects model **0.28 [-0.22; 0.78] 1.5%**
Heterogeneity: not applicable
Folic acid
Gagnon et al. (2018) 0.00 [-0.92; 0.92] 0.8%
Random effects model **0.00 [-0.92; 0.92] 0.8%**
Heterogeneity: not applicable
Ginseng
Ping et al. (2011) 0.38 [-0.55; 1.32] 0.8%
Random effects model **0.38 [-0.55; 1.32] 0.8%**
Heterogeneity: not applicable

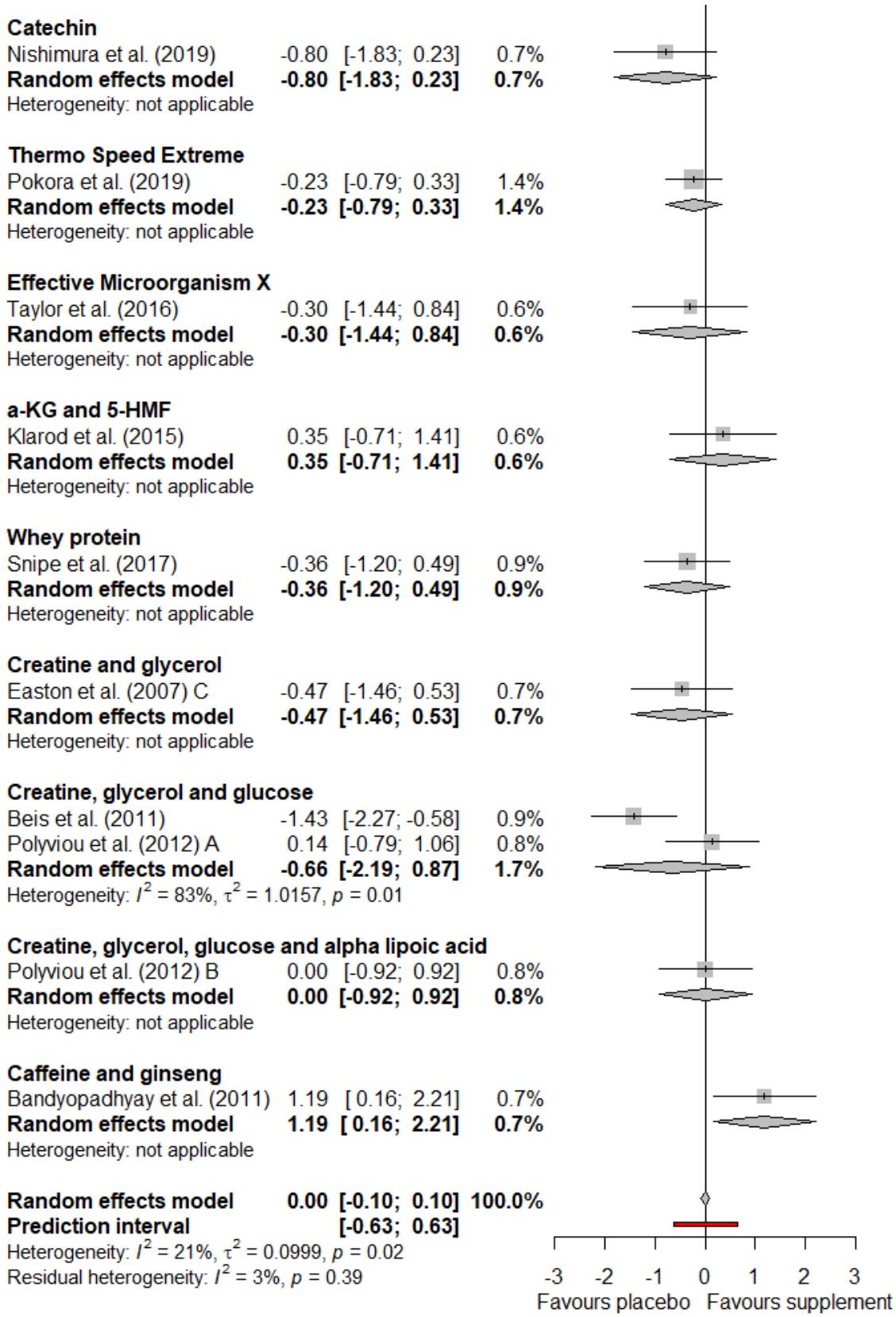
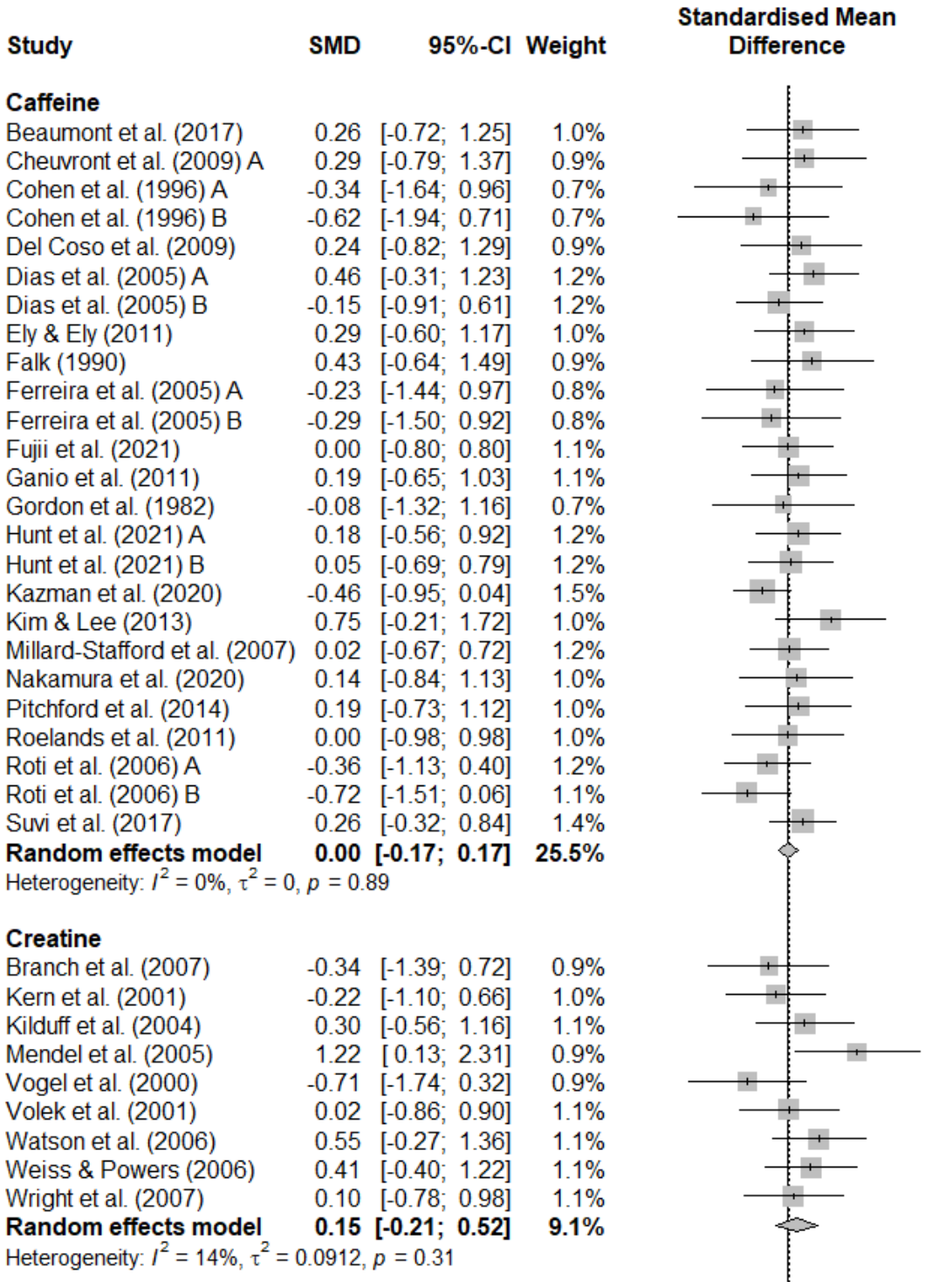
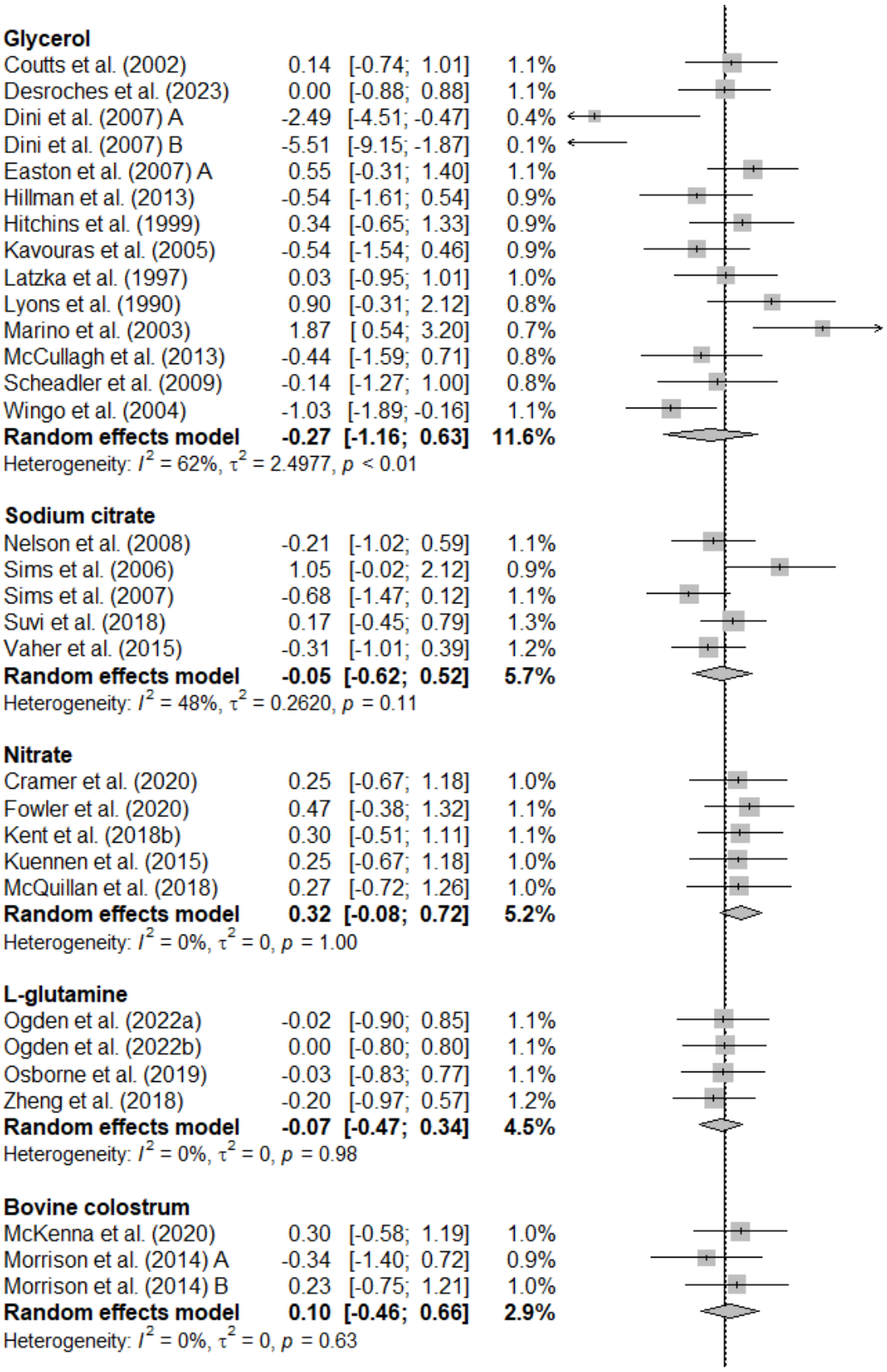
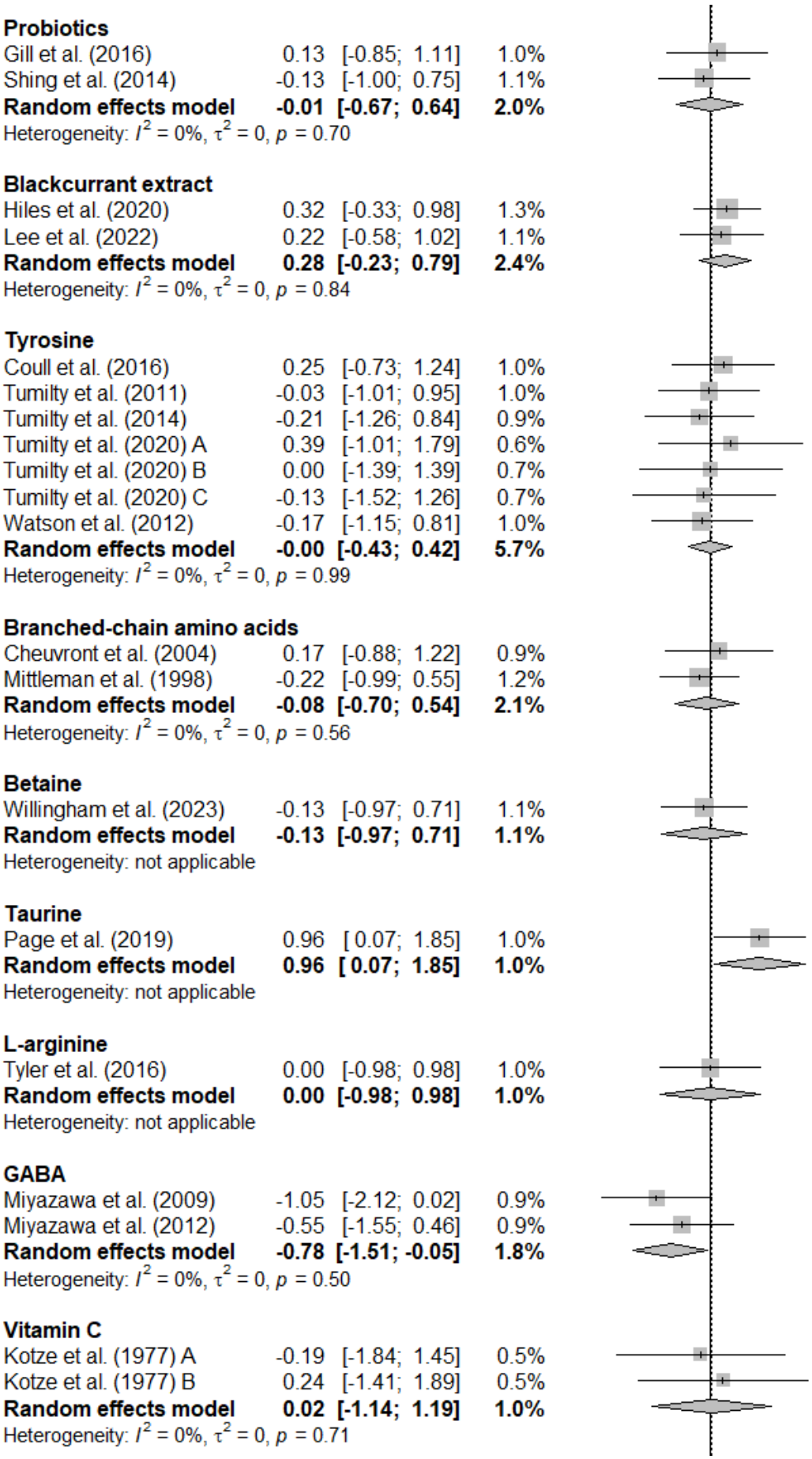
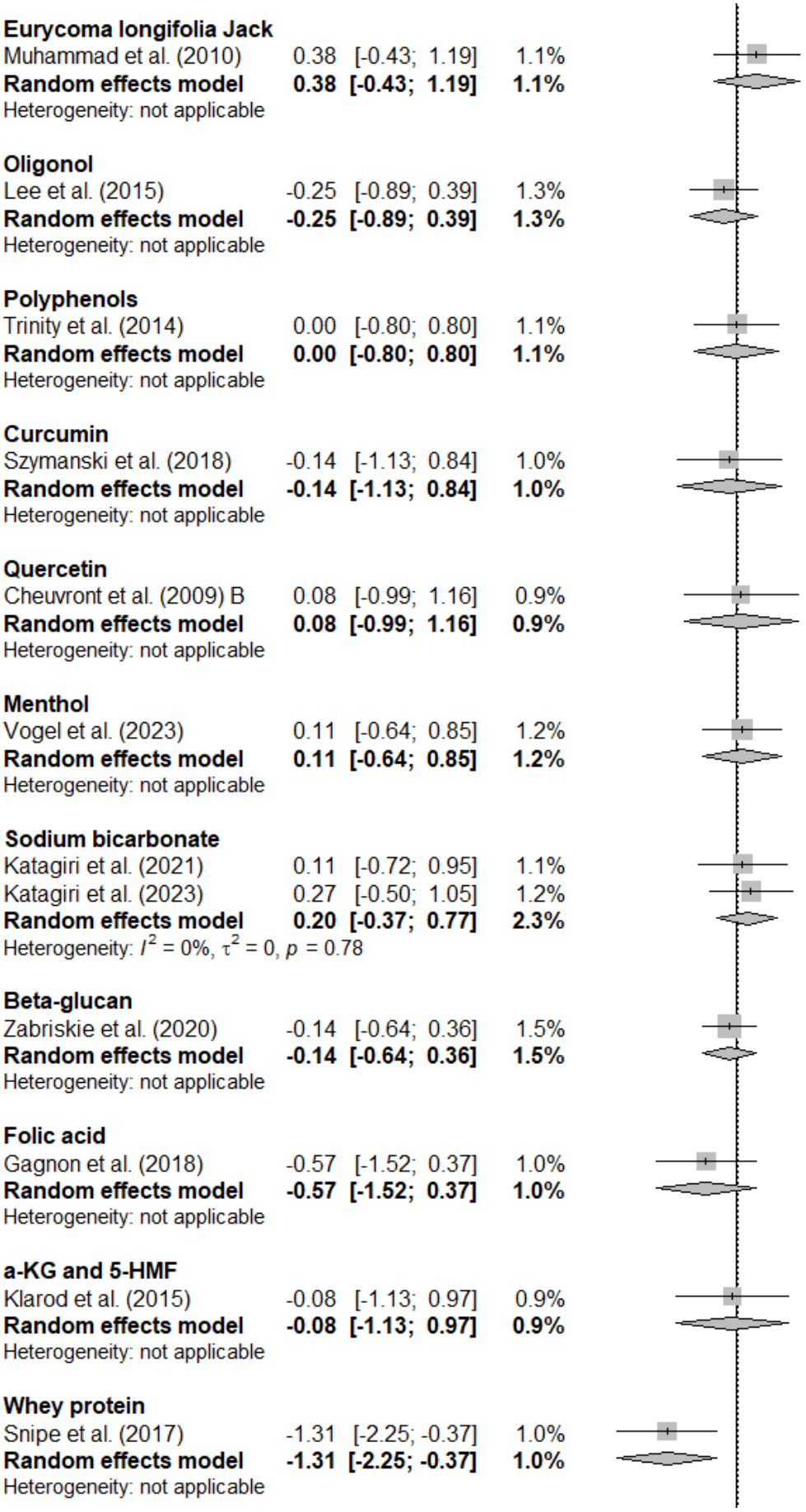


Figure 5.2. Effect of dietary supplementation on end core temperature.


Study
SMD
95%-CI
Weight
Standardised Mean Difference
Caffeine
Beaumont et al. (2017) 0.26 [-0.72; 1.25] 1.0%
Cheuvront et al. (2009) A 0.29 [-0.79; 1.37] 0.9%
Cohen et al. (1996) A -0.34 [-1.64; 0.96] 0.7%
Cohen et al. (1996) B -0.62 [-1.94; 0.71] 0.7%
Del Coso et al. (2009) 0.24 [-0.82; 1.29] 0.9%
Dias et al. (2005) A 0.46 [-0.31; 1.23] 1.2%
Dias et al. (2005) B -0.15 [-0.91; 0.61] 1.2%
Ely & Ely (2011) 0.29 [-0.60; 1.17] 1.0%
Falk (1990) 0.43 [-0.64; 1.49] 0.9%
Ferreira et al. (2005) A -0.23 [-1.44; 0.97] 0.8%
Ferreira et al. (2005) B -0.29 [-1.50; 0.92] 0.8%
Fujii et al. (2021) 0.00 [-0.80; 0.80] 1.1%
Ganio et al. (2011) 0.19 [-0.65; 1.03] 1.1%
Gordon et al. (1982) -0.08 [-1.32; 1.16] 0.7%
Hunt et al. (2021) A 0.18 [-0.56; 0.92] 1.2%
Hunt et al. (2021) B 0.05 [-0.69; 0.79] 1.2%
Kazman et al. (2020) -0.46 [-0.95; 0.04] 1.5%
Kim & Lee (2013) 0.75 [-0.21; 1.72] 1.0%
Millard-Stafford et al. (2007) 0.02 [-0.67; 0.72] 1.2%
Nakamura et al. (2020) 0.14 [-0.84; 1.13] 1.0%
Pitchford et al. (2014) 0.19 [-0.73; 1.12] 1.0%
Roelands et al. (2011) 0.00 [-0.98; 0.98] 1.0%
Roti et al. (2006) A -0.36 [-1.13; 0.40] 1.2%
Roti et al. (2006) B -0.72 [-1.51; 0.06] 1.1%
Suvi et al. (2017) 0.26 [-0.32; 0.84] 1.4%
Random effects model 0.00 [-0.17; 0.17] 25.5%
Heterogeneity: I^2 = 0%, τ^2 = 0, p = 0.89
Creatine
Branch et al. (2007) -0.34 [-1.39; 0.72] 0.9%
Kern et al. (2001) -0.22 [-1.10; 0.66] 1.0%
Kilduff et al. (2004) 0.30 [-0.56; 1.16] 1.1%
Mendel et al. (2005) 1.22 [0.13; 2.31] 0.9%
Vogel et al. (2000) -0.71 [-1.74; 0.32] 0.9%
Volek et al. (2001) 0.02 [-0.86; 0.90] 1.1%
Watson et al. (2006) 0.55 [-0.27; 1.36] 1.1%
Weiss & Powers (2006) 0.41 [-0.40; 1.22] 1.1%
Wright et al. (2007) 0.10 [-0.78; 0.98] 1.1%
Random effects model 0.15 [-0.21; 0.52] 9.1%
Heterogeneity: I^2 = 14%, τ^2 = 0.0912, p = 0.31


Glycerol
Coutts et al. (2002) 0.14 [-0.74; 1.01] 1.1%
Desroches et al. (2023) 0.00 [-0.88; 0.88] 1.1%
Dini et al. (2007) A -2.49 [-4.51; -0.47] 0.4%
Dini et al. (2007) B -5.51 [-9.15; -1.87] 0.1%
Easton et al. (2007) A 0.55 [-0.31; 1.40] 1.1%
Hillman et al. (2013) -0.54 [-1.61; 0.54] 0.9%
Hitchins et al. (1999) 0.34 [-0.65; 1.33] 0.9%
Kavouras et al. (2005) -0.54 [-1.54; 0.46] 0.9%
Latzka et al. (1997) 0.03 [-0.95; 1.01] 1.0%
Lyons et al. (1990) 0.90 [-0.31; 2.12] 0.8%
Marino et al. (2003) 1.87 [0.54; 3.20] 0.7%
McCullagh et al. (2013) -0.44 [-1.59; 0.71] 0.8%
Scheadler et al. (2009) -0.14 [-1.27; 1.00] 0.8%
Wingo et al. (2004) -1.03 [-1.89; -0.16] 1.1%
Random effects model -0.27 [-1.16; 0.63] 11.6%
Heterogeneity: I^2 = 62%, τ^2 = 2.4977, p < 0.01
Sodium citrate
Nelson et al. (2008) -0.21 [-1.02; 0.59] 1.1%
Sims et al. (2006) 1.05 [-0.02; 2.12] 0.9%
Sims et al. (2007) -0.68 [-1.47; 0.12] 1.1%
Suvi et al. (2018) 0.17 [-0.45; 0.79] 1.3%
Vaher et al. (2015) -0.31 [-1.01; 0.39] 1.2%
Random effects model -0.05 [-0.62; 0.52] 5.7%
Heterogeneity: I^2 = 48%, τ^2 = 0.2620, p = 0.11
Nitrate
Cramer et al. (2020) 0.25 [-0.67; 1.18] 1.0%
Fowler et al. (2020) 0.47 [-0.38; 1.32] 1.1%
Kent et al. (2018b) 0.30 [-0.51; 1.11] 1.1%
Kuennen et al. (2015) 0.25 [-0.67; 1.18] 1.0%
McQuillan et al. (2018) 0.27 [-0.72; 1.26] 1.0%
Random effects model 0.32 [-0.08; 0.72] 5.2%
Heterogeneity: I^2 = 0%, τ^2 = 0, p = 1.00
L-glutamine
Ogden et al. (2022a) -0.02 [-0.90; 0.85] 1.1%
Ogden et al. (2022b) 0.00 [-0.80; 0.80] 1.1%
Osborne et al. (2019) -0.03 [-0.83; 0.77] 1.1%
Zheng et al. (2018) -0.20 [-0.97; 0.57] 1.2%
Random effects model -0.07 [-0.47; 0.34] 4.5%
Heterogeneity: I^2 = 0%, τ^2 = 0, p = 0.98
Bovine colostrum
McKenna et al. (2020) 0.30 [-0.58; 1.19] 1.0%
Morrison et al. (2014) A -0.34 [-1.40; 0.72] 0.9%
Morrison et al. (2014) B 0.23 [-0.75; 1.21] 1.0%
Random effects model 0.10 [-0.46; 0.66] 2.9%
Heterogeneity: I^2 = 0%, τ^2 = 0, p = 0.63


Probiotics
Gill et al. (2016) 0.13 [-0.85; 1.11] 1.0%
Shing et al. (2014) -0.13 [-1.00; 0.75] 1.1%
Random effects model -0.01 [-0.67; 0.64] 2.0%
Heterogeneity: I^2 = 0%, τ^2 = 0, p = 0.70
Blackcurrant extract
Hiles et al. (2020) 0.32 [-0.33; 0.98] 1.3%
Lee et al. (2022) 0.22 [-0.58; 1.02] 1.1%
Random effects model 0.28 [-0.23; 0.79] 2.4%
Heterogeneity: I^2 = 0%, τ^2 = 0, p = 0.84
Tyrosine
Coull et al. (2016) 0.25 [-0.73; 1.24] 1.0%
Tumilty et al. (2011) -0.03 [-1.01; 0.95] 1.0%
Tumilty et al. (2014) -0.21 [-1.26; 0.84] 0.9%
Tumilty et al. (2020) A 0.39 [-1.01; 1.79] 0.6%
Tumilty et al. (2020) B 0.00 [-1.39; 1.39] 0.7%
Tumilty et al. (2020) C -0.13 [-1.52; 1.26] 0.7%
Watson et al. (2012) -0.17 [-1.15; 0.81] 1.0%
Random effects model -0.00 [-0.43; 0.42] 5.7%
Heterogeneity: I^2 = 0%, τ^2 = 0, p = 0.99
Branched-chain amino acids
Cheuvront et al. (2004) 0.17 [-0.88; 1.22] 0.9%
Mittleman et al. (1998) -0.22 [-0.99; 0.55] 1.2%
Random effects model -0.08 [-0.70; 0.54] 2.1%
Heterogeneity: I^2 = 0%, τ^2 = 0, p = 0.56
Betaine
Willingham et al. (2023) -0.13 [-0.97; 0.71] 1.1%
Random effects model -0.13 [-0.97; 0.71] 1.1%
Heterogeneity: not applicable
Taurine
Page et al. (2019) 0.96 [0.07; 1.85] 1.0%
Random effects model 0.96 [0.07; 1.85] 1.0%
Heterogeneity: not applicable
L-arginine
Tyler et al. (2016) 0.00 [-0.98; 0.98] 1.0%
Random effects model 0.00 [-0.98; 0.98] 1.0%
Heterogeneity: not applicable
GABA
Miyazawa et al. (2009) -1.05 [-2.12; 0.02] 0.9%
Miyazawa et al. (2012) -0.55 [-1.55; 0.46] 0.9%
Random effects model -0.78 [-1.51; -0.05] 1.8%
Heterogeneity: I^2 = 0%, τ^2 = 0, p = 0.50
Vitamin C
Kotze et al. (1977) A -0.19 [-1.84; 1.45] 0.5%
Kotze et al. (1977) B 0.24 [-1.41; 1.89] 0.5%
Random effects model 0.02 [-1.14; 1.19] 1.0%
Heterogeneity: I^2 = 0%, τ^2 = 0, p = 0.71


Eurycoma longifolia Jack
Muhammad et al. (2010) 0.38 [-0.43; 1.19] 1.1%
Random effects model **0.38 [-0.43; 1.19] 1.1%**
Heterogeneity: not applicable
Oligonol
Lee et al. (2015) -0.25 [-0.89; 0.39] 1.3%
Random effects model **-0.25 [-0.89; 0.39] 1.3%**
Heterogeneity: not applicable
Polyphenols
Trinity et al. (2014) 0.00 [-0.80; 0.80] 1.1%
Random effects model **0.00 [-0.80; 0.80] 1.1%**
Heterogeneity: not applicable
Curcumin
Szymanski et al. (2018) -0.14 [-1.13; 0.84] 1.0%
Random effects model **-0.14 [-1.13; 0.84] 1.0%**
Heterogeneity: not applicable
Quercetin
Cheuvront et al. (2009) B 0.08 [-0.99; 1.16] 0.9%
Random effects model **0.08 [-0.99; 1.16] 0.9%**
Heterogeneity: not applicable
Menthol
Vogel et al. (2023) 0.11 [-0.64; 0.85] 1.2%
Random effects model **0.11 [-0.64; 0.85] 1.2%**
Heterogeneity: not applicable
Sodium bicarbonate
Katagiri et al. (2021) 0.11 [-0.72; 0.95] 1.1%
Katagiri et al. (2023) 0.27 [-0.50; 1.05] 1.2%
Random effects model **0.20 [-0.37; 0.77] 2.3%**
Heterogeneity: I^2 = 0%, τ^2 = 0, p = 0.78
Beta-glucan
Zabriskie et al. (2020) -0.14 [-0.64; 0.36] 1.5%
Random effects model **-0.14 [-0.64; 0.36] 1.5%**
Heterogeneity: not applicable
Folic acid
Gagnon et al. (2018) -0.57 [-1.52; 0.37] 1.0%
Random effects model **-0.57 [-1.52; 0.37] 1.0%**
Heterogeneity: not applicable
a-KG and 5-HMF
Klarod et al. (2015) -0.08 [-1.13; 0.97] 0.9%
Random effects model **-0.08 [-1.13; 0.97] 0.9%**
Heterogeneity: not applicable
Whey protein
Snipe et al. (2017) -1.31 [-2.25; -0.37] 1.0%
Random effects model **-1.31 [-2.25; -0.37] 1.0%**
Heterogeneity: not applicable

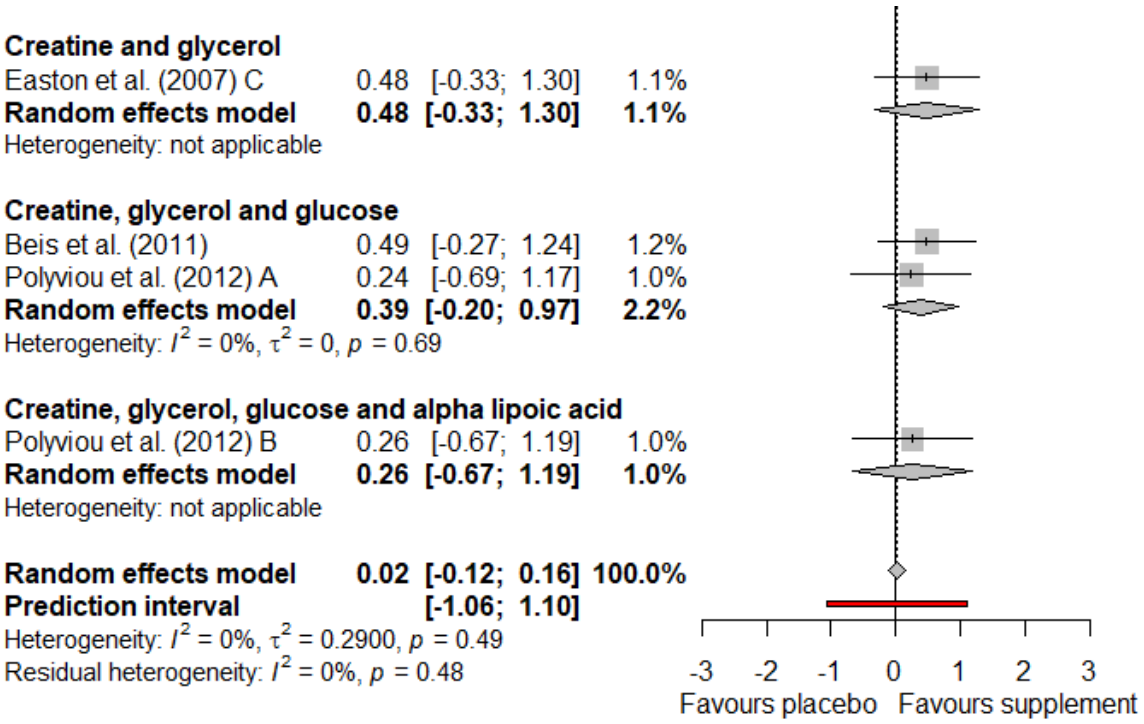


Figure 5.3. Effect of dietary supplementation on whole-body sweat rate.

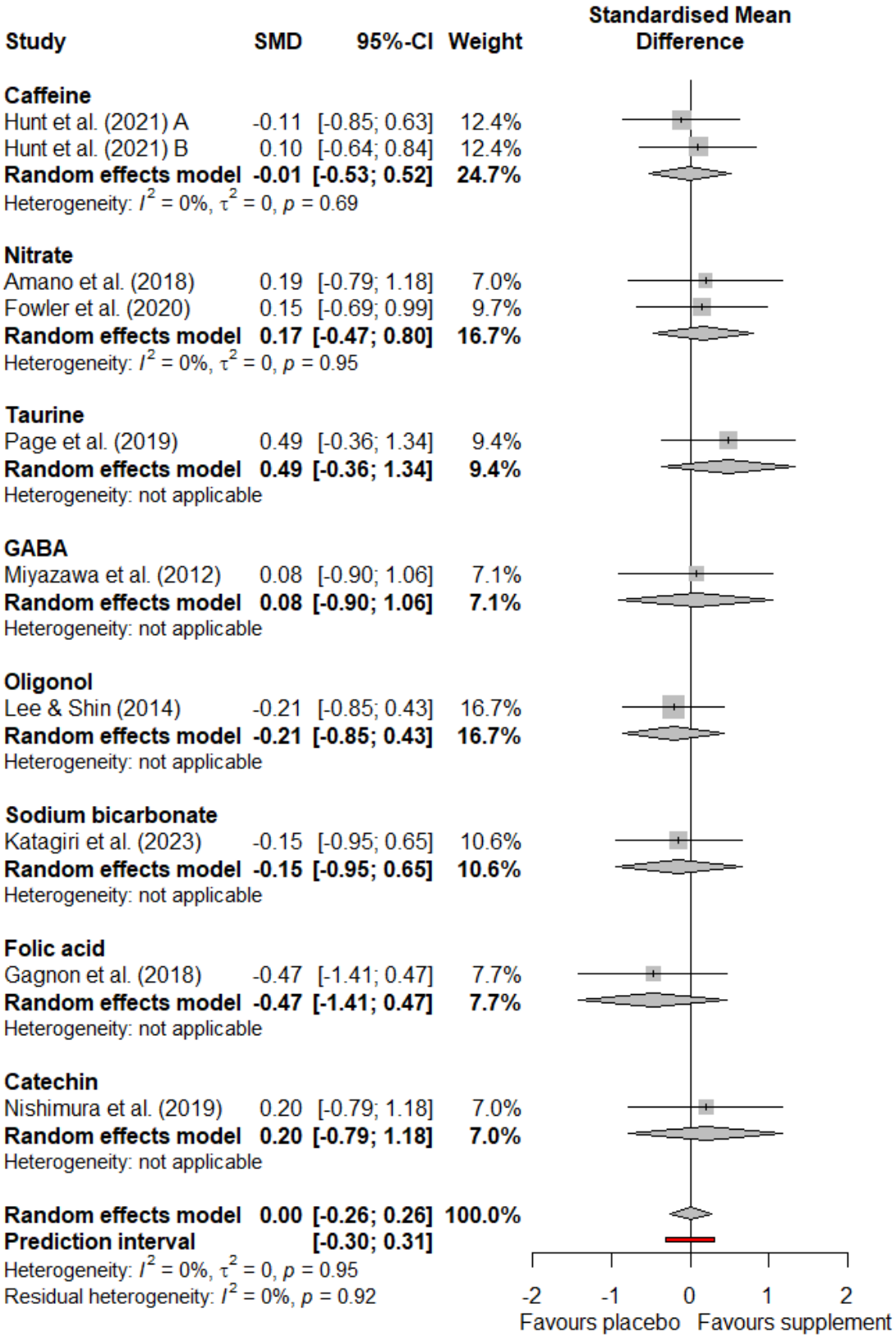


Figure 5.4. Effect of dietary supplementation on local sweat rate.

5.4.4. Sub-group analysis

5.4.4.1. End core temperature

Sub-group analysis demonstrated a significant overall pooled effect of the different dietary supplement categories on end T_{core} (p = 0.016). However, the following supplements all demonstrated non-significant *trivial* effects on end T_{core}: creatine (Hedges' g = -0.12, 95% CI -0.453-0.203, p = 0.456), nitrate (Hedges' g = 0.07, 95% CI -0.237-0.381, p = 0.648), L-glutamine (Hedges' g = 0.07, 95% CI -0.246-0.384, p = 0.667), bovine colostrum (Hedges' g = 0.13, 95% CI -0.327-0.588, p = 0.575), probiotics (Hedges' g = 0.00, 95% CI -0.653-0.653, p = 1.000), blackcurrant extract (Hedges' g = -0.11, 95% CI -0.461-0.453, p = 0.986), tyrosine (Hedges' g = -0.04, 95% CI -0.427-0.345, p = 0.835), BCAAs (Hedges' g = -0.004, 95% CI -0.327-0.588, p = 0.575), betaine (Hedges' g = -0.14, 95% CI -0.749-0.464, p = 0.646), vitamin C (Hedges' g = -0.07, 95% CI -0.891-0.743, p = 0.859), Eurycoma longifolia Jack (Hedges' g = 0.00, 95% CI -0.800-0.800, p = 1.000), polyphenols (Hedges' g = 0.00, 95% CI -0.800-0.800, p = 1.000), quercetin (Hedges' g = -0.24, 95% CI -0.973-0.496, p = 0.524), folic acid (Hedges' g = 0.00, 95% CI -0.924-0.924, p = 1.000) and creatine, glycerol, glucose and alpha lipoic acid (Hedges' g = 0.00, 95% CI -0.924-0.924, p = 1.000).

There were a number of caffeine-based supplements that increased T_{core}, with isolated caffeine (Hedges' g = 0.43, 95% CI 0.257-0.598, p < 0.001) having a *small* significant positive effect and combined caffeine and ginseng demonstrating a *large* significant positive effect (Hedges' g = 1.19, 95% CI 0.163-2.208, p = 0.023). L-arginine (Hedges' g = 0.22, 95% CI -0.765-1.203, p = 0.663), sodium bicarbonate (Hedges' g = 0.26, 95% CI -0.309-0.829, p = 0.370), beta-glucan (Hedges' g = 0.28, 95% CI -0.217-0.784, p = 0.268), ginseng (Hedges' g = 0.38, 95% CI -0.554-1.316, p = 0.424) and alpha-ketoglutaric acid (α-KG), 5-hydroxymethylfurfural (5-HMF) combined (Hedges' g = 0.35, 95% CI -0.709-1.408, p = 0.518) had *small* non-significant positive effects. Glycerol (Hedges' g = -0.28, 95% CI -0.700-0.130, p = 0.179), sodium citrate (Hedges' g = -0.46, 95% CI -1.261-0.341, p = 0.261), GABA (Hedges' g = -0.46, 95% CI -1.401-0.480, p = 0.337), vitamin E (Hedges' g = -0.23, 95% CI -0.889-0.423, p = 0.487), curcumin (Hedges' g = -0.28, 95% CI -1.268-0.704, p = 0.575), Thermo Speed Extreme (Hedges' g = -0.23, 95% CI -0.785-0.327, p = 0.420), Effective microorganism X (Hedges' g = -0.30, 95% CI -1.441-0.841, p = 0.606), whey protein (Hedges' g = -0.36, 95% CI -1.201-0.486, p = 0.407) and creatine and glycerol combined (Hedges' g = -0.47, 95% CI -1.460-0.530, p = 0.359) had *small* non-significant negative effects.

There were some medium-to-large effects of supplementation on end T_{core}, such as oligonol (Hedges' g = -0.50, 95% CI -0.907-0.101, p = 0.014), which had a *medium* significant negative

effect, and menthol (Hedges' *g* = -0.65, 95% CI -1.945-0.638, *p* = 0.321) and creatine, glycerol and glucose combined (Hedges' *g* = -0.66, 95% CI -2.187-0.873, *p* = 0.400) had *medium* non-significant effects. Both taurine (Hedges' *g* = -0.96, 95% CI -1.855-0.069, *p* = 0.035) and catechin (Hedges' *g* = -0.80, 95% CI -1.825-0.235, *p* = 0.130) had *large* negative effects on end T_{core}, but only taurine was significant.

5.4.4.2. Whole-body sweat rate

Sub-group analysis demonstrated a non-significant overall pooled effect of the different supplement categories on WBSR (*p* = 0.510). Caffeine (Hedges' *g* = 0.002, 95% CI -0.167-0.171, *p* = 0.979), creatine (Hedges' *g* = 0.15, 95% CI -0.208-0.517, *p* = 0.403), sodium citrate (Hedges' *g* = -0.05, 95% CI -0.616-0.525, *p* = 0.875), L-glutamine (Hedges' *g* = -0.07, 95% CI -0.473-0.337, *p* = 0.742), bovine colostrum (Hedges' *g* = 0.10, 95% CI -0.457-0.659, *p* = 0.723), probiotics (Hedges' *g* = -0.01, 95% CI -0.666-0.642, *p* = 0.972), tyrosine (Hedges' *g* = -0.001, 95% CI -0.426-0.423, *p* = 0.995), BCAAs (Hedges' *g* = -0.08, 95% CI -0.703-0.540, *p* = 0.797), betaine (Hedges' *g* = -0.13, 95% CI -0.967-0.706, *p* = 0.760), L-arginine (Hedges' *g* = 0.00, 95% CI -0.980-0.980, *p* = 1.000), vitamin C (Hedges' *g* = 0.02, 95% CI -1.141-1.191, *p* = 0.967), polyphenols (Hedges' *g* = 0.00, 95% CI -0.800-0.800, *p* = 1.000), curcumin (Hedges' *g* = -0.14, 95% CI -1.126-0.838, *p* = 0.774), quercetin (Hedges' *g* = 0.08, 95% CI -0.992-1.156, *p* = 0.881), menthol (Hedges' *g* = 0.11, 95% CI -0.635-0.848, *p* = 0.779), beta-glucan (Hedges' *g* = -0.14, 95% CI -0.635-0.362, *p* = 0.591) and α-KG and 5-HMF combined (Hedges' *g* = -0.08, 95% CI -1.131-0.965, *p* = 0.877) all had *trivial* non-significant effects.

For WBSR, nitrate (Hedges' *g* = 0.32, 95% CI -0.083-0.716, *p* = 0.120), blackcurrant extract (Hedges' *g* = 0.28, 95% CI -0.227-0.791, *p* = 0.277), Eurycoma longifolia Jack (Hedges' *g* = 0.38, 95% CI -0.429-1.188, *p* = 0.358), sodium bicarbonate (Hedges' *g* = 0.20, 95% CI -0.368-0.768, *p* = 0.490), creatine and glycerol combined (Hedges' *g* = 0.48, 95% CI -0.332-1.296, *p* = 0.246), creatine, glycerol and glucose combined (Hedges' *g* = 0.39, 95% CI -0.195-0.975, *p* = 0.192) and creatine, glycerol, glucose and alpha lipoic acid combined (Hedges' *g* = 0.26, 95% CI -0.671-1.187, *p* = 0.586) had *small* non-significant positive effects. Glycerol (Hedges' *g* = -0.27, 95% CI -1.163-0.632, *p* = 0.562) and oligonol (Hedges' *g* = -0.25, 95% CI -0.886-0.392, *p* = 0.448) had a *small* non-significant negative effect.

There were a number of medium-to-large effects on WBSR, including GABA (Hedges' *g* = -0.78, 95% CI -1.514- -0.053, *p* = 0.036), which had a *medium* significant negative effect and folic acid (Hedges' *g* = -0.57, 95% CI -1.523-0.373, *p* = 0.235), which had a *medium* non-significant negative effect. Taurine (Hedges' *g* = 0.96, 95% CI 0.065-1.850, *p* = 0.035) had a

large significant positive effect and whey protein (Hedges' *g* = -1.31, 95% CI -2.248-0.371, *p* = 0.006) had a *large* non-significant negative effect.

5.4.4.3. Local sweat rate

Sub-group analysis demonstrated a non-significant effect of the different supplement categories on LSR (*p* = 0.864). Caffeine (Hedges' *g* = -0.005, 95% CI -0.529-0.519, *p* = 0.985), GABA (Hedges' *g* = 0.08, 95% CI -0.905-1.056, *p* = 0.880), nitrate (Hedges' *g* = 0.17, 95% CI -0.471-0.804, *p* = 0.608) and sodium bicarbonate (Hedges' *g* = -0.15, 95% CI -0.950-0.653, *p* = 0.717) had *trivial* non-significant effects. Catechin (Hedges' *g* = 0.20, 95% CI -0.787-1.180, *p* = 0.695), and taurine (Hedges' *g* = 0.49, 95% CI -0.365-1.336, *p* = 0.263) had *medium* non-significant positive effects. Oligonol (Hedges' *g* = -0.21, 95% CI -0.849-0.427, *p* = 0.517) and folic acid (Hedges' *g* = -0.47, 95% CI -1.414-0.467, *p* = 0.323) also had *medium* non-significant effects but decreased the local sweating response.

5.4.5. Meta-regression

Across the three meta-analyses, there was only one significant ($p < 0.05$) moderating effect, which was the inverse association between glycerol dose and WBSR (Table 5.2), indicating that higher glycerol doses were related to lower sweating rates. The effect of several moderating variables on LSR could not be assessed due to either an insufficient number of studies included in the analysis (supplement dose) or a lack of variation within the moderating variables in the included studies (e.g. training, heat acclimation and hydration status).

Table 5.2. Meta-regression of potential moderating variables of the end core temperature, whole-body sweat rate and local sweat rate meta-analyses.

Moderator	End core temperature	Whole-body sweat rate	Local sweat rate
Training status	β = -0.021, p = 0.846 (n = 107)	β = -0.096, p = 0.564 (n = 84)	____
Heat acclimation status	β = 0.111, p = 0.560 (n = 66)	β = -0.415, p = 0.121 (n = 60)	____
Hydration status	β = -0.200, p = 0.754 (n = 100)	β = -0.150, p = 0.854 (n = 79)	____
Fluid ingestion during the trial	β = 0.001, p = 0.997 (n = 83)	β = -0.149, p = 0.448 (n = 68)	β = 0.111, p = 0.840 (n = 5)
Wet-bulb globe temperature	β = -0.007, p = 0.665 (n = 126)	β = -0.006, p = 0.810 (n = 99)	β = -0.006, p = 0.858 (n = 10)
Trial type	β = -0.247, p = 0.133 (n = 127)	β = -0.118, p = 0.691 (n = 101)	β = -0.229, p = 0.403 (n = 10)
Trial length	β = 0.001, p = 0.654 (n = 126)	β = -0.003, p = 0.200 (n = 100)	β = -0.005, p = 0.408 (n = 10)
Dosing duration	β = -0.005, p = 0.542 (n = 127)	β = -0.003, p = 0.826 (n = 101)	β = -0.014, p = 0.248 (n = 10)
Caffeine dose	β = 0.041, p = 0.429 (n = 28)	β = -0.044, p = 0.374 (n = 24)	____

Creatine dose	β = 0.057, p = 0.551 (n = 10)	β = 0.077, p = 0.448 (n = 9)	____
Glycerol dose	β = -0.340, p = 0.837 (n = 11)	β = -2.215, p = 0.032 (n = 14)	____
Sodium citrate dose	β = 0.003, p = 0.589 (n = 5)	β = -0.181, p = 0.911 (n = 5)	____
Nitrate dose	β = -0.006, p = 0.895 (n = 8)	β = -0.002, p = 0.967 (n = 5)	____
Glutamine dose	β = 0.015, p = 0.980 (n = 10)	β = -0.085, p = 0.919 (n = 4)	____
Tyrosine dose	β = -0.003, p = 0.183 (n = 8)	β = -0.001, p = 0.865 (n = 7)	____

5.4.6. Risk of bias

The studies included had a generally 'low' or 'unclear' risk of bias, with all but 11 studies stating randomisation procedures (Fowler et al., 2020; Hiles et al., 2020; Hunt et al., 2021; Lee et al., 2022; March et al., 2019; Ogden et al., 2022; Ogden et al., 2022; Page et al., 2019; Smith et al., 2019; Tumilty et al., 2020; Zabriskie et al., 2020) and three studies with pre-post intervention designs not randomising or blinding (Beis et al., 2011; Cramer et al., 2020; Gagnon et al., 2018). Allocation concealment was 'high' in three studies (Beis et al., 2011; Cramer et al., 2020; Gagnon et al., 2018; Figure 5.5). A number of outliers were detected in the end T_{core} meta-analysis (Bandyopadhyay et al., 2011; Beis et al., 2011; Cheuvront et al., 2009; Ely et al., 2011; Lyons et al., 1990; Millard-Stafford et al., 2007; Ping et al., 2010; Riera et al., 2014; Roelands et al., 2011; Sims et al., 2007; Zuhl et al., 2014), owing to the large effects that were elicited by some supplements on end T_{core} responses and Egger's test showed that there was no publication bias (p = 0.510). Several outliers were detected in the WBSR meta-analysis (Dini et al., 2007; Marino et al., 2003; Snipe et al., 2017; Wingo et al., 2004); however, Egger's test indicated no publication bias (p = 0.425) and influence analysis demonstrated no outcome changes when these were removed. No outliers or publication bias (p = 0.358) were detected in the LSR meta-analysis (Figure 5.6).

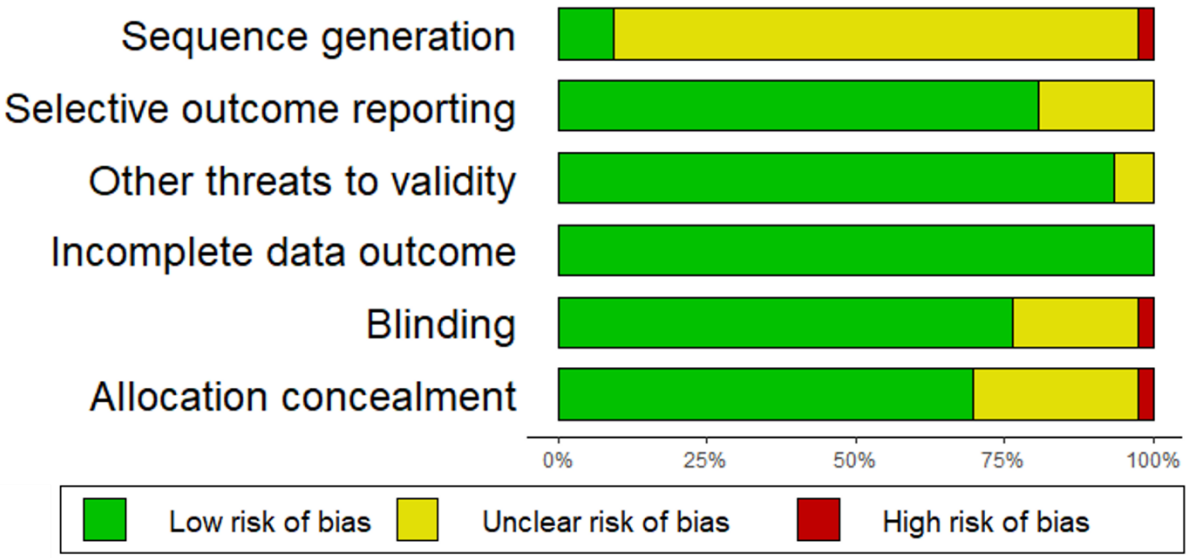


Figure 5.5. Risk of bias.

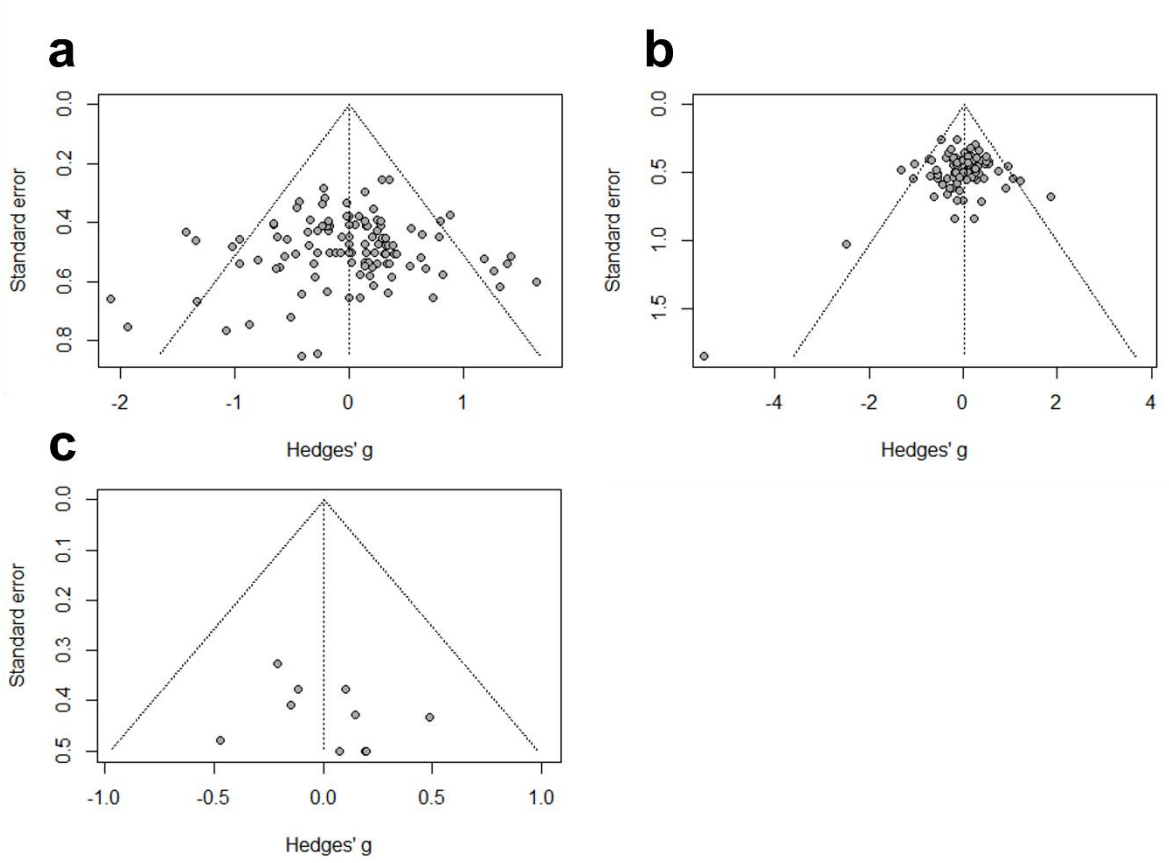


Figure 5.6. Publication bias for (a) end core temperature, (b) whole-body sweat rate and (c) local sweat rate.

5.5. Discussion

The main findings of the current meta-analyses were that, overall, pooled analysis of all dietary supplements demonstrated no effect on end T_{core} in the heat (Figure 5.2). However, there were differences between supplements, with caffeine, taurine and oligonol significantly affecting end T_{core} responses but not in consistent ways. Caffeine supplementation appeared to induce a thermogenic effect, while other supplements, such as taurine and oligonol lowered T_{core} responses compared to placebo. This is consistent the previous meta-analytical findings within this thesis on the thermal effects of caffeine and taurine during exercise in the heat (Chapter Four), but this work has now expanded the evidence to a wider pool of supplements, across both resting and exercising conditions. Further, the additional analyses of sweating responses revealed that, collectively dietary supplements may increase WBSR (Figure 5.3), but have limited effects on LSR, which is likely to be due to the smaller number of studies included in the analysis (Figure 5.4). Despite this, there was variation across supplements in regard to their effects on sweating, with taurine, demonstrating the greatest increases in WBSR and LSR, and others such as oligonol and folic reducing these responses. In the heat, sweating is

the primary heat loss avenue, and as such, is responsible for limiting thermal gain (i.e. T_{core} increases). The findings, herein, which indicate that dietary supplements may influences these aspects of thermoregulation, have implications for individuals exposed to hot environmental conditions. Further, as demonstrated by the meta-regression analysis (Table 5.2), there were also no moderating effects of training and heat acclimation status, hydration status, fluid ingestion during the trial and WBGT on the supplements' capacity to alter T_{core} and sweating responses.

5.5.1. Nitrate, L-arginine and folic acid

In regard to WBSR and LSR, the current analysis revealed a *small* and *trivial*, non-significant positive effect of nitrate, which is a supplement well-established to improve NO bioavailability (Lundberg et al., 2008; Lundberg et al., 2011). It is theorised that NO may contribute to eccrine sweat gland function, as local inhibition of NO synthase - NO's precursor - with N^G-nitro-L-arginine methyl ester (L-NAME), has been shown to attenuate sweat rate during moderate exercise in the heat (Fujii et al., 2014; Stapleton et al., 2014a), though not all studies provide support for this (Fujii et al., 2014). Further, NO also appears to have a role in regulating cutaneous vasodilation (Kellogg et al., 1998; McNamara et al., 2014; Shastry et al., 1998). Interestingly, numerous studies, in isolation, reported no significant increases in sweating (Amano et al., 2018; Cramer et al., 2020; Fowler et al., 2020; Kent, et al., 2018; Kuennen et al., 2015; McQuillan et al., 2018), which equated to a *small* non-significant effect based on the collective evidence of the current meta-analysis. Therefore, the degree to which nitrate supplementation augments the sweating response is likely to be insufficient to elicit substantial thermoregulatory benefits. This is supported by the findings of the end T_{core} analysis herein, where increases in sweating did not translate to reductions in T_{core}. These findings, in combination, therefore question whether nitrate supplementation has the capacity to aid thermal balance in hot environmental conditions. In line with this, supplementation with other precursors to NO, specifically L-arginine and folic acid, demonstrated no significant thermoregulatory improvements, as end T_{core} was not lower and sweat rate was not increased following their supplementation. Together, these results suggest that supplementation strategies aiming to increase NO bioavailability fail to enhance the sweating responses, and thus, opportunity to evaporatively cool when used in a hot environment. Interestingly, many of these studies also demonstrated no greater cutaneous blood flow or dry heat transfer capacity, thereby questioning any thermoregulatory advantage of nitrate or NO donors in the heat (Fowler et al., 2021; Gagnon et al., 2018; Tyler et al., 2016). Consequently, supplementation with ~4.2 to 16.8 mmol dietary nitrate, 10 g L-arginine or 5 mg folic acid cannot currently be

recommended during heat exposure to improve thermal balance, but do not appear to have any deleterious effects.

5.5.2. Caffeine

The end T_{core} analysis demonstrated that caffeine and caffeine in combination with ginseng had *small* (Hedges' g = 0.43, p < 0.001) and *large* (Hedges' g = 1.19, p = 0.023) significant positive effects, respectively. Substantial rises in T_{core} can cause heat strain and, ultimately, lead to heat exhaustion and heat stroke if not sufficiently controlled (Liu et al., 2022; Székely et al., 2015). This is particularly the case during heat exposure, or exercise in hot conditions, when avenues of dry heat dissipation are reduced due to a smaller temperature gradient between the ambient air and skin's surface (Che Muhamed et al., 2016; Kenny et al., 2018). There is evidence that caffeine supplementation increases $\dot{V}O_2$ and, consequently, heat production at comparable exercise intensities compared to placebo (Bell & McLellan, 2002; Ely et al., 2011), potentially explaining this observed T_{core} rise. Indeed, the $\dot{V}O_2$ response to exercise has been reported to be increased by 3% to 15% following caffeine supplementation in the heat (Falk et al., 1990; Millard-Stafford et al., 2007; Ping et al., 2010; Pitchford et al., 2014), although others have demonstrated negligible differences (Beaumont & James, 2017; Cheuvront et al., 2009; Roti et al., 2006). These results could be attributed to the methodological differences between studies, as noted by John et al. (2024), which calls for improved standardisation of laboratory procedures in studies relating to thermoregulation and caffeine supplementation. In addition to the current results, regarding T_{core}, WBSR and LSR were not increased with caffeine supplementation. Given the capacity of sweating to help dissipation of excess metabolic heat through evaporative cooling, these *negligible* effects would place greater demand upon dry heat transfer. However, it appears that caffeine-mediated reductions in cutaneous blood flow, owing to vasoconstriction of the skin microvasculature, would preclude this possibility (Daniels et al., 1998; Hunt et al., 2021). Thus, greater heat production, coupled with reduced SkBF and no increase in sweat production, explains why heat retention would ensue and the observed rises in T_{core} reported herein. Indeed, the previous meta-analytical findings support this observation and have been corroborated further by a recent meta-analysis, which demonstrated a 0.1°C/h greater T_{core} rate of rise following caffeine supplementation (Naulleau et al., 2022). Overall, this suggests that caffeine has an undesirable (i.e. heat gaining) effect on thermal balance and questions its use in hot environmental conditions. We, therefore, do not recommend acute caffeine intake of between 3 to 9 mg/kg prior to exercise, or at rest during heat exposure, due to its

thermogenic effect in such conditions, as this could result in an increased risk of heat related illnesses.

5.5.3. Taurine

The current evaluation of sweating responses revealed that taurine had a *large*, significant positive effect on both end T_{core} (Hedges' *g* = -0.96, *p* = 0.035) and WBSR (Hedges' *g* = 0.96, *p* = 0.035), as well as a *small,* non-significant positive effect on LSR. This single study suggests that ingestion of taurine prior to exercise in the heat augmented the sweating response by reducing sweat onset and increasing sweat rate (Page et al., 2019). Theoretically, this would improve thermal balance, due to enhanced latent heat transfer and reduced heat storage, delaying rises in T_{core} (Cramer & Jay, 2016; Gagge & Gonzalez, 1996). While this indicates that taurine can exert a beneficial thermoregulatory effect, these findings require replication, including further insight into its mechanisms of action, which are poorly understood. More thorough elucidation of taurine's effects on sweating and the consequential impact on heat transfer and heat tolerance is necessary, alongside investigation of these mechanisms. However, based on this study alone, acute intake of 50 mg/kg of taurine prior to exercise in the heat induces an earlier sweating onset, greater sweating rate and lower T_{core} response and may offer a strategy to improve thermoregulatory capacity.

5.5.4. Tyrosine, BCAAs and GABA

Tyrosine and BCAAs demonstrated no significant effect on end T_{core} or WBSR. Despite these amino acids previously being reported to provide some of the greatest performance effects of any supplements during exercise in the heat (Chapter Four), they do not appear to confer thermoregulatory benefits. The ability to reduce central fatigue is often ascribed to these supplements to explain their erogenicity in such conditions (Newsholme & Blomstrand, 1995; Nybo, 2008; Nybo et al., 2011; Tumilty et al., 2011), but no apparent link to temperature regulation was found in the current study. However, tyrosine is an essential substrate for tyrosine hydroxylase, which is involved in axonal catecholamine synthesis (particularly norepinephrine; Fernstrom & Fernstrom, 2007). Thus, sufficient tyrosine availability is required to maintain catecholamine levels and facilitate sympathetic vasoconstrictive effects on the subcutaneous vasculature (Lang et al., 2020). This has been reported to attenuate the rate of cold-induced decline in T_{core} among those likely to have tyrosine deficiency (Lang et al., 2020), but there were no similar effects reported across studies conducted in the heat. Interestingly, another amino acid, GABA, has some potential to offer thermoregulatory benefits, with the

end T_{core} analysis revealing a *small*, yet non-significant thermolytic effect. While one of the two studies that supplemented GABA demonstrated null end T_{core} effects, there was a slower rate of rise in T_{core} across the 30 minute exercising period (Miyazawa et al., 2012). GABA is a widely distributed neurotransmitter within the central nervous system, where it acts in the hypothalamus to regulate internal body temperature (Quéva et al., 2003; Watanabe et al., 2002; Yakimova et al., 1996). Exogenous supplementation in humans is thought to increase GABA's availability in the hypothalamus (Cavagnini et al., 1980) and, thereby, influence temperature regulation (Miyazawa et al., 2012). The hypothalamus contains cold-sensitive neurons, which have a role in controlling heat production upon detection of local and peripheral temperature changes (Hori, 1991; Nakayama, 1985). In the animal model, suppression of these neurons appears to occur following GABA administration, leading to lowered T_{core} responses in the heat (Ishiwata et al., 2005). Indeed, the two original research articles included in the current meta-analysis (Miyazawa et al., 2012; Miyazawa et al., 2009) observed reductions in metabolic heat production, which could in part explain the effects on T_{core} in the GABA supplementation conditions. Additionally, GABA attenuates activity of the sympathetic nervous system (Deuchars et al., 2005; Wible et al., 1989), which would likely supress epinephrine and norepinephrine release (Goldstein et al., 1983), as observed by Miyazawa et al. (2009). Previously, reductions in catecholamines have been associated with slower rises in T_{core} during hyperthermic exercise (Mora-Rodriguez et al., 1996), which is supported by the findings herein. Considering these effects on heat production, it is unsurprising that there was also a *large*, significant reduction in WBSR (Hedges' g = -0.78, p = 0.036) and a *trivial* effect on LSR, as it is a known driver of the thermal sweating response (Cramer & Jay, 2014, 2016; Chapter Six). Therefore, while GABA appears to reduce one avenue of heat dissipation (i.e. evaporative cooling), it has created a greater heat storage capacity, which would delay the onset of hyperthermic symptoms during heat stress and may be effective during short duration exercise in the heat. Based on the two studies herein, the administration of 1 g of GABA directly prior to heat exposure (rest or exercise) appears to provide a beneficial effect on thermal balance through a reduction in heat gain. However, acute tyrosine and BCAA intake is not suggested in hot environmental conditions, due to the null impacts on thermoregulation.

5.5.5. Glycerol and creatine

The end T_{core} analysis herein revealed *small*, non-significant negative effects for glycerol and combined creatine and glycerol supplementation. *Medium*, non-significant negative effects were observed for combined creatine, glycerol and glucose and *trivial*, non-significant effects for creatine and combined creatine, glycerol, glucose and alpha lipoic acid. While these results

demonstrate that glycerol supplementation had a *small*-to-*medium* effect on end T_{core}, the variation across studies decreased the certainty of these findings, rendering them non-significant. When ingested, glycerol provides osmotic pressure in the plasma and intra- and extra-cellular water compartments – where concentrations are evenly distributed – and thereby increase their water content (Jardine et al., 2023; Nelson & Robergs, 2007; Suvi et al., 2018). Creatines acts similarly, as its transport into cells (primarily skeletal muscle) increases total body water through expansion of intra- and extracellular water compartments, with even fluid distribution (Persky et al., 2003; Powers et al., 2003; Watson et al., 2006). This increase to total body water and plasma volume expansion induces 'hyperhydration' and affords excess fluid to compensate for sweat losses (Anderson et al., 2001; Coutts et al., 2002), alongside providing greater availability to sweat glands to facilitate sweat production (Nielsen et al., 1984). This would likely improve thermoregulatory capacity, through increased evaporative cooling, but also because additional total body water enhances the specific heat carrying capacity of the tissues and blood (Kay & Marino, 2000; Kilduff et al., 2004; Sawka, 1992). Here, it can help transfer heat from the core to the periphery to be dissipated (Chato, 1980; Keller & Seiler, 1971; Morimoto, 1990).

Creatine had a *trivial*, non-significant positive effect on WBSR and combined creatine and glycerol and combined creatine and glycerol with the addition of glucose and glucose and alpha lipoic acid had *small*, non-significant positive effects. The role of glycerol combined with creatine was, therefore, also partially effective in promoting a sweating response but, as with the end T_{core} responses, the effects were inconsistent across studies, which increased the uncertainty of the *small* effects. Surprisingly, glycerol alone had a *small*, non-significant negative effect on WBSR. Though, this was largely influenced by two glycerol supplementation conditions from the same study, where much larger fluid losses in the placebo group were reported (Dini et al., 2007). Collectively, it appears that these supplements may be capable of lowering T_{core} and enhancing sweating responses compared to placebo, with the combination of creatine and glycerol potentially providing the greatest thermoregulatory benefit. However, with the large inconsistencies between studies and non-significant findings this is far from established and further work is required to understand the heterogeneity of responses. This could be related to dose, as for glycerol, this was a significant moderating effect on WBSR (β = -2.215, p = 0.032). Herein, it is suggested that a greater glycerol dose was associated with a reduction in WBSR. Indeed, Dini et al. (2007) B provided the highest glycerol dose and observed a large, negative effect on WBSR (Hedges' g = -5.51). Theoretically, glycerol ingestion of a large quantity may surpass concentrations that can be absorbed into the intra- and extra-cellular fluid, further elevating plasma concentrations and increasing osmotic pressure. This may prevent fluid being drawn from the plasma to the sweat glands, thereby

decreasing sweat rate. However, without further investigation into the effect of glycerol dose on the sweating response, more specifically, this remains speculative. These findings demonstrate that lower doses of glycerol (1 to 1.4 g/kg), alone or in combination with 20 to 25 g/day of creatine for between 3 to 9 days, appear to aid thermal balance during exercising heat stress, through hyperhydration. Additional investigation into whether this supplementation strategy would provide similar benefits during passive heating is also warranted. Higher doses of glycerol (e.g. 3 g/kg), however, may reduce this capacity due to lower sweat rates, though a greater understanding is necessary before providing definitive recommendations.

5.5.6. Sodium citrate and sodium bicarbonate

As supplements often ingested prior to high-intensity exercise to improve blood buffering capacity, both sodium citrate and sodium bicarbonate have also been reported to increase plasma osmolality and plasma volume (Siegler et al., 2021). This could feasibly help with thermoregulation in the same manner as creatine or glycerol loading; indeed, ingestion of sodium citrate had a *small*, yet non-significant negative effect on end T_{core}, which was similar to the previous supplements detailed above. However, this was not coupled with a greater WBSR. Conversely, sodium bicarbonate had a *small*, non-significant positive effect on T_{core}. In these two studies, the placebo group ingested sodium chloride to match the sodium content of the two conditions, as they were investigating the buffering capacity of the supplement and not its effects on fluid balance (Katagiri et al., 2023; Katagiri et al., 2021). Therefore, it is likely that any potential osmotic effects that could theoretically have aided thermoregulation, would be indistinguishable from the effects in the placebo condition. In support of this, there was a *small*, non-significant positive effect on WBSR and no effect on LSR. To establish whether sodium bicarbonate's effects on fluid balance can aid thermoregulatory function in hot environmental, studies would need to be conducted with a placebo group that does not contain any sodium. It appears that sodium citrate can potentially improve thermoregulatory capacity in the heat, though this was inconsistent across studies. Any thermolytic effect is likely due to its effects on plasma volume, as expansion was observed across all studies included in the analysis, but to a larger degree in the studies which demonstrated lower T_{core} responses. As there was no greater WBSR associated with the lower T_{core} response, a greater heat carrying capacity of the blood may be responsible for these observed effects (Kay & Marino, 2000; Sawka, 1992). However, further research is required to corroborate these findings and establish whether sodium bicarbonate can elicit the same benefits. Therefore, based on the studies included in this analysis, there is evidence to suggest that acute sodium citrate ingestion of ~100 to 600 mg/kg can improve thermoregulatory capacity during exercise in the

heat, but more research is needed to establish these effects at rest and with sodium bicarbonate supplementation.

5.5.7. Betaine

Betaine is an amino acid, which acts as both an osmolyte, to assist with cell volume regulation, and as a methyl group donor to convert homocysteine to methionine (Lever & Slow, 2010). It can be supplemented to reduce high plasma concentrations of homocysteine (McRae, 2013) or to improve endurance and resistance exercise performance (Cholewa et al., 2014). Due to its osmotic role, it has mechanistic potential to aid fluid balance and thermoregulation during exposure to heat stress (Willingham et al., 2023). However, both the current end T_{core} and WBSR analyses demonstrated no effects. In addition to the measured variables in the current meta-analysis, one of the included studies observed plasma volume expansion across the study in response to betaine supplementation (Armstrong et al., 2008); however, the other did not (Willingham et al., 2023). Together, these results question whether betaine is efficacious for fluid balance when ingested prior to exercise in the heat. There is also some evidence to suggest that betaine may attenuate thermal cellular stress in a similar manner to heat shock proteins (Dangi et al., 2016; Willingham et al., 2020) and in the animal model it has repeatedly been demonstrated to reduce T_{core} when chronically supplemented (Attia et al., 2009; DiGiacomo et al., 2016; Zulkifli et al., 2004). Therefore, betaine may have the capacity to improve heat tolerability, and considering the limited and equivocal evidence in humans, this supplement requires further investigation.

5.5.8. Anti-oxidants and anti-inflammatories

In the current meta-analysis, lower end T_{core} responses for several supplements with known anti-oxidative and anti-inflammatory properties were found. Oligonol and catechin, had a *medium*, significant (Hedges' g = -0.50, p = 0.014) and a *large*, non-significant (Hedges' g = -0.80, p = 0.130) negative effect on end T_{core}, respectively. Furthermore, *small*, non-significant negative effects were observed for curcumin, vitamin E and Effective microorganism X (an anti-oxidant mixture) and *trivial*, non-significant negative effects for blackcurrant extract. The anti-inflammatory role of oligonol, catechins, curcumin, vitamin E, Effective microorganism X is most likely responsible for the lowered T_{core} responses compared to placebo, where endogenous pyrogenic cytokines (Vybíral et al., 2005) may be attenuated. Indeed, oligonol supplementation lowered circulating levels of the pyrogenic cytokines, such as interleukins IL1-β and IL-6 (Shin et al., 2011), along with reductions in serum prostaglandin E_2, a known

intermediary in the development of fever (Coceani et al., 1986; Shin et al., 2013). The cytokine response can be acutely lowered with anti-inflammatory substances (Moreland, 2009), theoretically leading to decreased thermal gain (Bradford et al., 2007), explaining why the rate of rise in T_{core} was reduced, despite no greater WBSR or LSR. However, not all of these studies observed reductions in pro-inflammatory cytokines (Lee et al., 2022; Szymanski et al., 2018), despite attenuated increases in T_{core}. All trials investigating oligonol and catechin – which had the greatest effects – induced heat strain via hot water immersion of the lower body at rest. This is a rapid means by which to facilitate heat gain, as water is highly conductive, yet it partially attenuates other avenues of heat dissipation, such as evaporation (Gagge & Gonzalez, 1996). It is possible that an immersive protocol induced greater thermal strain and production of pyrogenic cytokines, meaning that T_{core} responses were more readily identified between conditions. However, the rise in T_{core} within these trials was less than would be expected, only reaching an average of 37.52°C across all trials. Although this was with tympanic measurement, which may explain the relatively low T_{core} values. Another explanation for lower end T_{core} responses is increased heat dissipation, however, sweating was only greater following catechin and blackcurrant supplementation and these effects were non-significant. While this may partly explain the improved thermal balance in this instance, it appears likely that reductions in endogenous pyrogenic cytokines have an important role to play in the efficacy of many of the anti-inflammatory supplements. Nevertheless, only oligonol has demonstrated significant impacts on aspects of thermoregulation and, therefore, further examination of these supplements and mechanisms is required to provide definitive answers.

An additional role of anti-oxidants is to improve cellular oxidative capacity and, therefore, redox status (Gulcin, 2020). These effects could be directly extended to sudomotor function, based on the reported relationships between systemic markers of lipid peroxidation and sweat production (Hoeldtke et al., 2011). However, further research is needed to explore this possibility, owing to the failure of local anti-oxidant infusion to acutely alter the local sweating response during exercise-heat stress (Fujii et al., 2022), which questions the likelihood that anti-oxidants play a major role in thermoregulatory sweating. Indeed, a greater sweating response was not observed for the majority of these supplements. A component of catechin, epicatchin, has been associated with greater cutaneous blood flow during heat exposure through improved NO signalling (Brossette et al., 2011; Schroeter et al., 2006). The results were non-significant, but if substantiated, the observed augmented sweating response may be due to the associated enhancement of skin blood demonstrated in response to catechin supplementation (Nishimura et al., 2019). In combination, these would improve evaporative and dry heat transfer, explaining the lower T_{core} response. Similarly, there is evidence that anthocyanins, a key component of blackcurrants promotes production of NO, through

augmented NO synthase activity (Matsumoto et al., 2005). Furthermore, Eurycoma longifolia Jack – another supplement with an anti-oxidative function – had a *medium*, positive non-significant effect on WBSR, yet no significant change in end T_{core}. Neither of these two latter studies measured or estimated SkBF or characterised other indices of vascular function and, therefore, it can only be speculated that any potential greater WBSR – albeit non-significant – observed is in response to the aforementioned mechanisms.

Interestingly, beta-glucan and ginseng, other supplements with anti-inflammatory properties, had *small*, non-significant positive effects on end T_{core}, and beta-glucan also had no effect on WBSR. While other endogenous pyrogens were significantly reduced immediately post-exercise in the beta-glucan condition compared to placebo, there was a transient elevation of macrophage inflammatory protein 1β, which may provide a potential explanation for these findings. However, without further investigation into beta-glucans thermoregulatory effects during heat exposure, any mechanistic explanations remain speculative. Ginseng is a herb with many bioactive ingredients, which has been demonstrated to increase body temperature in the animal model, and may also partially explain this thermogenic effect (Park et al., 2014).

Other anti-oxidants; vitamin C and polyphenols had no observable effects on end T_{core} or WBSR. Quercetin and combined α-KG and 5-HMF also had no effect on WBSR, but a *small*, non-significant negative and a *small*, non-significant positive effect on end T_{core} was observed, respectively. It has been theorised that quercetin, a well characterised anti-oxidant, is capable of inhibiting (via ROS scavenging) the necessary molecular signalling events required to acquire the acclimated phenotype, by reducing the heat-shock factor or hypoxia-inducible factor response to heat exposure (Kuennen et al., 2011). Acutely, anti-oxidant intake would improve redox balance and potentially aid heat tolerance, but if supplemented chronically or alongside heat exposure may blunt adaptations (Kuennen et al., 2011; Pastor & Tur, 2019). A similar argument can be posed for supplements with anti-inflammatory properties (Lilja et al., 2018). Indeed, a greater number of studies demonstrated beneficial end T_{core} and sweating responses (e.g. catechin, oligonol, quercetin), when supplemented acutely (1 day), but there is no clear consensus on dosing length and supplement efficacy within the current analysis. Nevertheless, based on the required time-course of these cellular adaptations, this mechanism could partially explain the lack of difference between anti-oxidants consumed over longer periods (≥ 7-days) and placebo supplements in the current meta-analysis. Across the anti-oxidant and anti-inflammatory supplements in the current meta-analysis, 10 of the 19 supplements were consumed repeatedly across 3 to 42 days, which means that any potential thermoregulatory effects may have been masked. In summary, the use of anti-oxidants results in variable responses in the heat, which could be partly explained by their multi-ingredient composition, or dosing period. It is important that the specific mechanisms by which these

variable effects occur should be investigated, especially if chronic administration of anti-oxidants and anti-inflammatories may reduce adaptation to heat exposure and exacerbate heat illness. Indeed, many of these supplements are more likely to be ingested by people who require anti-oxidative or anti-inflammatory agents, such as older or clinical populations, who are also more vulnerable to heat stress (Kenny et al., 2010; Vandentorren et al., 2006) and are also less likely to tolerate increases in T_{core} (i.e. ginseng). Such individuals could benefit from reductions in T_{core} and dietary supplements that may induce this (i.e. oligonol and catechin), assuming that there are no other apparent side-effects.

The current analysis suggests that 100 to 200 mg oligonol ingested approximately 30 to 60 min pre-heat exposure has a beneficial effect on thermal balance, by reducing heat gain. Although further investigation into oligonol's efficacy during exercising heat stress is necessary to further elucidate its effects on thermoregulatory capacity. Catechin appears to have a similar effect, though corroboration of this finding is required, as only one study has been conducted thus far. There is also tentative evidence that prolonged intake of 800 mg curcumin, vitamin E, 70 ml Effective microorganism X (3, 42 and 7 days, respectively), may reduce T_{core} responses and 600 mg blackcurrant extract and 150 mg Eurycoma longifolia Jack may improve sweat rate during exercise in the heat. However, these results were non-significant and based on only one (curcumin, vitamin E, Effective microorganism X and Eurycoma longifolia Jack) or two studies (blackcurrant extract), so these results are not conclusive. Further research is required to establish these anti-inflammatory supplements efficacy during heat exposure and their effects on endogenous pyrogenic cytokines. Additional investigation into their effects when ingested acutely, at rest and during more ecological valid conditions is warranted before more definitive thermoregulatory effects can be established. Similarly, the potential thermogenic effects of 200 mg ginseng, 250 mg beta-glucan and combined 4.8 g α-KG and 60 mg 5-HMF require additional examination, as their intake cannot currently be recommended based on the results herein. Further, ingestion of 250 to 1500 mg vitamin C and polyphenols does not appear to influence thermoregulatory responses (T_{core} or sweat rate) during exercising heat stress and, therefore, while intake is unlikely to facilitate improved thermal balance, it is also unlikely to have detrimental performance or heath consequences. However, establishing these effects following acute doses may reveal further impacts on thermoregulatory capacity.

5.5.9. L-glutamine, bovine colostrum, probiotics and whey protein

L-glutamine, bovine colostrum and probiotic supplementation all had no effects on end T_{core} and WBSR, suggesting that they confer no thermoregulatory benefit in the heat. These

supplements, along with curcumin, are often ingested prior to exercise in hot environmental conditions, with the aim of maintaining gastrointestinal (GI) barrier integrity and reducing symptoms of GI dysfunction. Gastrointestinal injury and changes to epithelial permeability are relatively common during exercising heat stress (Chantler et al., 2021), which consequently, leads to translocation of endotoxins and bacterial lipopolysaccharides into the central circulation, causing systemic endotoxaemia (Bosenberg et al., 1988). The subsequent release of pro-inflammatory cytokines can, in turn, cause cytokaemia and additional elevations in T_{core} (Bosenberg et al., 1988; Lim & Mackinnon, 2006; Selkirk et al., 2008). However, evidence for these supplements' efficacy is equivocal, along with their function as ergogenic aids in the heat. Favourable effects of whey protein supplementation have been demonstrated on GI permeability during exercising heat stress (Snipe et al., 2017), where there was also a *small*, non-significant negative effect on end T_{core} and a *large*, significant negative effect on WBSR (Hedges' g = -1.31, p = 0.006). The whey protein condition had a lower circulatory endotoxin concentration post-exercise compared with placebo, which theoretically may explain any T_{core} differences. The large inhibitory effect on sweating was unexpected, but given the lower T_{core} – and likely heat production -, the drive for sweating would be reduced (Gagnon et al., 2013). The only other supplement to display any potential improvements to thermoregulatory capacity is curcumin – as detailed previously – which is more likely to be due to its aforementioned anti-inflammatory role. Probiotics and bovine colostrum supplementation did not reduce circulating endotoxin or cytokine concentrations in the studies within which these were measured (Gill et al., 2016; Morrison et al., 2014; Osborne et al., 2019; Shing et al., 2014) and only one study which supplemented L-glutamine demonstrated reductions in endotoxins and TNF-α (Zuhl et al., 2015). These supplements may be less effective at preventing GI injury in the heat, due to greater redistribution of blood flow from the gut (GI ischaemia) to the peripheral vasculature (Yeh et al., 2013) and, consequently, have no influence of T_{core} responses. Indeed, only a few studies identified improvements to GI barrier integrity (March et al., 2019; Pugh et al., 2017; Zuhl et al., 2015; Zuhl et al., 2014) and largely attributed this improvement to upregulation of heat shock protein 70, which inhibits inflammation (Nava et al., 2019; Zuhl et al., 2015; Zuhl et al., 2014). Therefore, the long-term use of probiotics (7 to 28 days) and ~20 to 140 g bovine colostrum (7 to 14 days) and acute use of 0.15 to 0.9 g/kg L-glutamine to maintain GI barrier integrity in hot environmental conditions appears to provide no thermoregulatory advantage. Whilst supplements targeting the GI tract during heat stress are an area of ongoing interest, further research is required in this area to establish other efficacious alternatives. Indeed, an acute dose of whey protein (15 g) may provide an effective option (Snipe et al., 2017), but without replication of these results, this cannot be definitively stated.

5.5.10. Menthol and Thermo Speed Extreme

The oral supplementation of menthol non-significantly lowered end T_{core}. This large reduction was unanticipated but the variability across studies explains the non-significant effect. Menthol is typically considered to be a non-thermal cooling agent (Barwood et al., 2020), which evokes the perception of cooling via transduction of the TRPM8 receptors in the oral cavity (Andersen et al., 2014; Liu et al., 2020; McKemy et al., 2002; Peier et al., 2002) and possibly the viscera (Harrington et al., 2011), without directly affecting thermal balance according to current literature (Flood et al., 2017; Jeffries et al., 2018; Jeffries & Waldron, 2019). However, these findings were heavily influenced by a single study's results, where T_{core} was estimated by tympanic measurement (Riera et al., 2014), which can be less reliable if the correct procedures are not adhered to. Therefore, there is some doubt over these results. As discussed in Barwood and colleagues' expert-led consensus article, there are some reports of menthol causing vaso-reactivity in the skin's subcutaneous vasculature when applied externally, but no consensus was reached on any form of menthol administration and thermoregulatory effects. Therefore, replication of this single study may be required to confirm whether acute menthol ingestion can mechanistically affect temperature regulation. Additionally, there was no effect on WBSR, which supports the current consensus (Barwood et al., 2020). Thermo Speed Extreme is another supplement which did not affect end T_{core} and given that this supplement contains ingredients such as caffeine (Pokora et al., 2019), this finding is somewhat unexpected. It is possible that the tympanic measures used within this study were too insensitive to identify T_{core} changes. However, significantly greater chest T_{sk} were observed at certain time-points across the trial, which could enhance dry heat dissipation to the environment, particularly as the ambient air was much cooler (26.2°C) than average T_{sk} across participants (34°C). The ingredient piperine could be responsible for this likely enhancement of cutaneous vasodilation, as *in-vitro* work suggests it has vaso-modulating effects (Taqvi et al., 2008). This could explain the tendency towards lower T_{core} values, despite the thermogenic nature of the supplement. It should be stated that this supplement would, therefore, not be appropriate for use in ambient temperatures that exceed T_{sk}, where skin surface to ambient air temperature gradients, and dry heat transfer capacity are reduced.

5.5.11. Moderating effects

No candidate moderators, such as training and heat acclimation status, hydration status and fluid ingestion during the trial, affected end T_{core} or sweating responses to dietary supplementation. For hydration status, this is likely due to the majority of papers stipulating the inclusion of hydrated participants. However, there was more variation in the training (highly

trained; 43% *vs* recreationally active; 39%) and heat acclimation (acclimated; 14% *vs* non-acclimated; 36%) status of participants and whether fluid was ingested during the trials (ingested; 46% *vs* not ingested; 22%), yet no moderating effects were found. Nevertheless, it would be useful for future studies to consider investigating the efficacy of dietary supplements on thermoregulation among participants of different training and heat acclimation statuses, given the effect of these processes on sweating and T_{core} responses (Ravanelli et al., 2018). Some supplements, such as sodium citrate, nitrate, L-glutamine and tyrosine, have only been used to assess thermoregulatory responses in non-acclimated participants, which limits the wider application of these to potential end-users. Whether this would augment or negate any effects seen from these supplements is of particular interest and important to establish for individuals in competitive sport, military and occupational settings. In addition, all other meta-regressions (WBGT, trial type and length and supplementation period) did not moderate the effect of dietary supplementation on end T_{core} or sweating. There is a large range of supplements included within the current meta-analyses, each with differing underpinning mechanisms and nuances in their efficacy. It is, therefore, unsurprising that there are no consistent moderating factors.

While these trial moderators had no significant effects in the present analysis, they still require further investigation, particularly within the most efficacious supplements included here. For example, acute supplementation and the use of exercise was most common, with no variation within certain supplement categories. The effect of chronic supplementation of certain supplements, such as various anti-oxidants, glycerol, taurine, and other amino acids (e.g. L-glutamine), on T_{core} and sweating responses in the heat is almost completely unknown. Taurine has been shown to elicit various physiological effects following chronic supplementation, which may be advantageous during heat exposure, such as enhanced vascular function (Sun et al., 2016) and an improved endurance trained phenotype (Ahmadian et al., 2017; Lee et al., 2003; Zhang et al., 2004). All studies investigating the effects of L-glutamine on GI barrier integrity in the heat have supplemented acutely and it is possible that a chronic dose may be more efficacious. Indeed, long-term administration (2 months) has demonstrated beneficial effects on GI permeability in patients with Crohn's disease (Benjamin et al., 2012). Longer term glycerol intake has previously elicited hyperhydration for up to 49 hours (Koenigsberg et al., 1995), but whether it can be maintained over a greater period of time is currently unknown. Further research into this, alongside potential side effects (e.g. hyponatraemia) would help establish whether glycerol supplementation can provide long-term beneficial effects on thermal tolerance. Additionally, as detailed above, the chronic and acute effects of various anti-oxidants and anti-inflammatory supplements in the heat requires investigation. Furthermore, establishing the efficacy of these supplements during differing trial types with

differing physiological demands is necessary to be able to provide practical advice and application to athletes, workers and the general population. The meta-regression of WBGT demonstrated no effect, but ambient vapour pressure does have an established impact on avenues of heat dissipation (Che Muhamed et al., 2016). For example, supplements which augment thermal sweating (e.g. taurine) will likely be most effective in dry environments where any sweat produced can be evaporated, thereby providing a cooling effect. Depending on the mechanistic actions of certain supplements, beneficial thermoregulatory effects, this could have a large impact on their efficacy and ability to help individuals maintain thermal equilibrium. Investigation of these above factors is important, particularly amongst the most efficacious of the supplements examined within this meta-analysis.

Limitations

Within this meta-analysis, several supplements were taken in combination, such as creatine and glycerol (Easton et al., 2007 C; Beis et al., 2011; Polyviou et al., 2012 A & B), caffeine and ginseng (Bandyopadhyay et al., 2011), combined α-KG and 5-HMF (Klarod et al., 2015), whey protein (Snipe et al., 2017), Effective microorgansim X (Taylor et al., 2016) and Thermo Speed Extreme (Pokora et al., 2019). However, as only a few studies employed a co-ingestion strategy, there is limited information on the thermoregulatory outcomes when using this approach across a wide range of different supplements. Herein, the combined effect of creatine and glycerol was beneficial for thermal balance, while the co-ingestion of caffeine and ginseng further exacerbated caffeine's thermogenic effect. As such, the former could be recommended to improve fluid balance in the heat; however, the latter poses a greater heat stress risk and should be avoided in hot conditions. This is perhaps unsurprising given that caffeine alone causes an increase in T_{core}. Considering these differing findings and the indication that co-ingestion potentially influences the thermoregulatory responses to these supplements, greater clarity across supplement types regarding these effects is certainly warranted. Indeed, athletes and military personnel often combine dietary supplements (Baylis & Cameron-Smith, 2001; Casey et al., 2014), which may increase the risk of heat stress if a harmful combination is unwittingly ingested. Therefore, further research regarding the effect of dietary supplement co-ingestion on thermoregulatory responses during heat exposure is necessary and represents a key gap in the literature and a further lack of specific supplementation guidance for potential users.

5.5.12. Conclusion

In summary, for the first time, the effects of various dietary supplements on T_{core} and sweating responses in the heat have been evaluated. The amino acids taurine and GABA, alongside whey protein, lowered end T_{core}, indicating an improvement to thermal balance. While GABA and whey protein negatively impacted WBSR, taurine increased the sweating response, demonstrating an enhancement to thermoregulatory capacity, albeit from a single study. However, other amino acids, such as tyrosine and BCAAs appeared to have no meaningful effect on thermoregulation. Various supplements with anti-oxidative and anti-inflammatory properties (e.g. oligonol, catechin, curcumin, vitamin E and quercetin) provided beneficial effects on end T_{core}, which may in-part be explained through improved redox balance and attenuation of endogenous pyrogenic cytokines. Nevertheless, not all of these supplements improved thermal balance, highlighting the need for additional research in this area. A number of supplements (e.g. glycerol, creatine, sodium citrate and betaine), which appear to induce hyperhydration and/or expand plasma volume, non-significantly lowered T_{core} responses. Mechanistically, this may be through increasing heat carrying capacity and/or improving fluid availability to the sweat gland, as some supplements (e.g. combined glycerol and creatine) also demonstrated an effect on sudomotor function. However, T_{core} and sweat rate findings were inconsistent across studies and supplement types, rendering the results non-significant overall. Many other supplements such as nitrate, L-arginine, folic acid (taken for their effects on NO bioavailability) and L-glutamine, bovine colostrum and probiotics (taken for their effects on GI barrier integrity) did not appear to provide any thermoregulatory benefit in the heat. End T_{core} was greater following caffeine and combined caffeine and ginseng supplementation, without any increases in sweating responses. Consequently, caffeine ingestion during heat exposure may increase the risk of heat related illnesses and have potential negative health implications. Several other supplements, such as ginseng, beta-glucan and combined α-KG and 5-HMF also demonstrated *small* thermogenic effects, though these results were non-significant.

Although additional investigation is certainly required, some supplements have demonstrated the potential to improve thermoregulatory capacity in the heat. However, it appears that others have null or even deleterious effects on thermal balance when ingested in such conditions. These findings suggest that certain supplements, such as caffeine, should be avoided in hot conditions and others, such as taurine, may elicit a thermoregulatory benefit. This has potential implications for those ingesting dietary supplements for their health and/or performance effects during periods of heat exposure. Indeed, official guidance documents for the general population, athletes and military personnel could also be updated to reflect the varying effects different dietary supplements appear to have on thermoregulation, detailing which to avoid

and which may be advantageous in hot conditions. Additional investigation into many of these supplements is required to corroborate findings and provide greater understanding of their effects. Specifically, future research should focus on the thermolytic effects of various supplements such as taurine, GABA, oligonol and catechin in varying conditions, alongside further mechanistic insight into these responses.

6. Chapter Six – Measurement of thermal sweating at rest and steady-state exercise in healthy adults: Inter-day reliability and relationships with components of partitional calorimetry

The study that comprises Chapter Six has been published in Plos one. Chapter Six is identical to the published version, with the only adaptations due to required formatting alterations and changes to several figure legends.

Reference: Peel, J. S., McNarry, M. A., Heffernan, S. M., Nevola, V. R., Kilduff, L. P., & Waldron, M. (2022). Measurement of thermal sweating at rest and steady-state exercise in healthy adults: Inter-day reliability and relationships with components of partitional calorimetry. *Plos one*, *17*(12), e0278652.

6.1. Abstract

Background Valid and reliable measurements of the thermal sweating response to exercise are important for accurate estimations of evaporative cooling and detection of heat acclimation. Indeed, changes in measures of thermal sweating, such as LSR (~30%) and SGA (~27.9%) have been reported following heat acclimation. Therefore, these measures, and their determining factors ($\dot{H}_{prod}$ and $\dot{E}_{req}$), are fundamental to the investigation of human thermoregulation.

Objectives Inter-day reliability of sweat measurements, including the absorbent patch and modified iodine-paper techniques, at rest and exercise were evaluated. The effect of iodine paper size and the method of establishing SGA (sweat gland counting or surface area covered) on reliability were also established. Furthermore, the relationships between all measurement techniques and $\dot{H}_{prod}$ and $\dot{E}_{req}$ were determined.

Methods Twelve participants were assessed for WBSL, LSR (absorbent patch) and SGA (iodine-paper) during rest and sub-maximal cycling at ~200, ~250 and ~300 W/m^2 $\dot{H}_{prod}$ in the heat. Variations in iodine paper (1 x 1 cm-9 x 9 cm) were used to quantify SGA by counting sweat glands or surface area covered. The 'optimal' area of SGA was also determined based on the highest density of recruited glands.

Results All measures of the sweating response were positively related with $\dot{H}_{prod}$ and $\dot{E}_{req}$ (r = 0.53 to 0.84), with the 9 x 9 cm and 6 x 6 cm iodine paper sizes being the strongest (r = 0.66 to 0.84) for SGA. Superior inter-day reliability was found for all measures during exercise (CV% = 6 to 33.2) compared to rest (CV% = 33.5 to 77.9). The iodine-paper technique was

most reliable at 9 x 9 cm (CV% = 15.9) or when the 1 x 1 cm (CV% = 17.6) and 3 x 3 cm (CV% = 15.5) optimal SGA was determined, particularly when measuring the sweat gland number.

Conclusion WBSL, LSR and SGA measurement techniques are sufficiently reliable to detect changes in thermal sweating typically reported. This study recommends 9 x 9 cm paper sizes or 1 x 1 cm to 3 x 3 cm optimal areas, using either gland counting or surface area to determine SGA.

6.2. Introduction

Evaporation of fluid off the skin's surface represents the greatest avenue of heat loss during exercise in hot environments (Nielsen, 1938); eccrine sweat production and evaporation is, therefore, the most important physiological mechanism for the maintenance of heat balance in such conditions. Indeed, the requirement for evaporative cooling to dissipate excess heat from the body in order to maintain heat balance ($\dot{E}_{req}$), by definition, drives the steady-state sweating response (Cramer & Jay, 2014, 2016; Gagnon et al., 2013). This is supported by a recent study which found that steady-state sweating was similar across a range of absolute T_{core} and T_{sk} conditions that elicited the same $\dot{E}_{req}$ (Ravanelli et al., 2020). Similarly, metabolic heat production ($\dot{H}_{prod}$) is also positively related to the rate of whole-body sweating (Hospers et al., 2020), which is intuitive as $\dot{E}_{req}$ is determined as the net difference between $\dot{H}_{prod}$ and the sum of respiratory and dry heat exchange (Cramer & Jay, 2019). Therefore, both $\dot{H}_{prod}$ and $\dot{E}_{req}$ are two modifiable variables responsible for driving the rate of evaporative cooling, which can be estimated using partitional calorimetry in a controlled laboratory setting (Cramer & Jay, 2019). Valid and reliable measurements of thermal sweating during exercise, and their determining factors ($\dot{H}_{prod}$ and $\dot{E}_{req}$), are fundamental to the investigation and interpretation of human thermoregulation, yet the reliability of these measures has not been sufficiently reported.

There are numerous ways to assess sweating responses in humans (Baker, 2017; Morris et al., 2013), with many methods designed to determine WBSR or LSR (Baker et al., 2018) and SGA (Gagnon et al., 2012). Local sweat rate is typically measured using the ventilated capsule method (Baker et al., 2018); additionally, absorbent patches affixed to the skin can be used by assessing pre- to post-exercise differences in patch mass, across a known time period (Baker, 2017; Baker et al., 2018; Smith & Havenith, 2011). This is a long-standing technique, which has been adapted from early work in the 1930/40s (Ogata, 1935; Weiner, 1945), leading to the more recent technical absorbent method (Havenith et al., 2008; Smith & Havenith, 2011, 2012a) and absorbent patch technique (Baker, 2017; Baker et al., 2018). The technical absorbent method was strongly associated with the ventilated capsule method (r = 0.74 to

0.95), across varying time durations and patch sizes (sample surface area) and locations (body region; Morris et al., 2013). Estimated WBSL is most commonly reported by comparing changes in body mass pre- versus post-exercise (Armstrong, 2007; Cheuvront & Kenefick, 2011). Sweat gland activation can be assessed using the modified iodine-paper technique, which is a manual way of measuring sweat droplets on the skin surface and can subsequently be quantified using a computer programme. This technique produces repeatable, intra-trial values (coefficient of variation [CV%] = 11 ± 10%) during controlled exercising conditions (Gagnon et al., 2012). However, this method does not account for clustering of sweat droplets from multiple glands. This could affect the consistency of results and might be resolved by expressing SGA according to the surface area covered, which has not been previously reported in the literature. Lastly, an important element of this technique is the application of iodine impregnated paper onto the skin surface. Varying sizes of iodine paper have been used in different studies and are reported inconsistently (Madeira et al., 2010; Moyen et al., 2014; Park et al., 2020; Poirier et al., 2016; Ravanelli et al., 2018). It is possible that the size of the paper significantly affects the repeatability of this manual technique, which assumes an even distribution of SGA across the measured areas. Thus, the effect of the paper size on measurement error requires further investigation.

While intra-trial (i.e. within a single trial day, recorded seconds-to minutes apart) reliability studies have been conducted for some methods, such as the modified iodine-paper technique (Gagnon et al., 2012) and the absorbent patch technique for measuring LSR (Baker et al., 2018), none have analysed their inter-day reliability at rest and a range of relative exercise intensities. Considering that the majority of studies have multiple testing sessions, spanning several days (or weeks) and exercise intensity varies across studies, it is important to appreciate the variability under such differing circumstances and the subsequent reliability of the technique. In addition, there are daily fluctuations in individuals' physiological responses to stimuli, which could potentially create noise when assessments are conducted across a longer time period (Kenefick et al., 2012). Without quantifying the reliability (i.e. noise) of a technique across several days, its efficacy (e.g. detection limits) is unknown, and thus the capacity to identify meaningful changes in sweat (i.e. rate and SGA) as a result of an intervention, such as acclimation or dietary supplementation, cannot be determined (Hopkins, 2000). Furthermore, the reliability of these techniques should be considered in conjunction with the established drivers of thermal sweating ($\dot{H}_{prod}$ and $\dot{E}_{req}$) since error in sweating measures will be partly determined by variation in these factors.

On the understanding that $\dot{H}_{prod}$ and $\dot{E}_{req}$ predominantly determine the magnitude of required evaporative cooling during exercise in hot environments, (Gagnon et al., 2013; Ravanelli et al., 2020) it stands to reason that any measurement used to determine the sweating response

should be positively associated, and therefore constructively valid. This includes measures such as SGA, LSR and WBSL. Indeed, $\dot{H}_{prod}$ and $\dot{E}_{req}$ have been positively associated to WBSR and LSR, determined using absorbent patches (Bain et al., 2011). The relationship between $\dot{E}_{req}$ and WBSR, measured via the ventilated capsule method has also been established (Gagnon et al., 2013; Hospers et al., 2020; Ravanelli et al., 2020). However, the relationship between $\dot{H}_{prod}$ and $\dot{E}_{req}$ and all other techniques for assessing the sweating response have not.

Based on the above reasoning, the aims of the current study were to establish the inter-day reliability of: i) the modified iodine-paper technique for the measurement of SGA using two separate assessment methods (sweat gland counting and surface area covered); ii) the absorbent patch technique for the measurement of LSR; and iii) pre- *vs* post-exercise body mass changes for measurement of WBSL. Finally, the relationship between all measurements of thermal sweating and both $\dot{H}_{prod}$ and $\dot{E}_{req}$ was assessed to establish the construct validity of these measurements.

6.3. Methods

6.3.1. Participants

Twelve, non-heat acclimated, healthy, recreationally active, females (*n* = 5) and males (*n* = 7) volunteered for the study (29 ± 6 years, 175.0 ± 7.6 cm, 76.5 ± 11.6 kg). Participants were asked to refrain from alcohol, avoid strenuous exercise and follow a consistent diet for 24 h prior to testing. Use of any dietary supplement was prohibited for the duration of the study. Written informed consent was obtained from all participants. Institutional ethical approval was provided for this study (JP_25-11-20; Appendix A), which was conducted in accordance with the 2013 Declaration of Helsinki.

6.3.2. Design

Participants reported to the laboratory on three occasions, across three separate days. The first visit comprised of preliminary testing and familiarisation; the inter-day test-retest reliability trials were subsequently conducted on the second and third visits. Specifically, during visits 2 and 3, the participants completed a discontinuous, sub-maximal cycling protocol at exercise intensities designed to elicit three 30 min stages of incremental heat production ($\dot{H}_{prod}$; ~200, ~250 and ~300 W/m^2) whilst exposed to ambient heat stress. Each stage was separated by 10 min of rest. During each stage sweat-related measurements were conducted for

subsequent assessment of their reliability between days. All visits took place in a temperature-controlled room at 37.6 ± 0.4°C and 27.0 ± 5.9% RH (mean ± SD). To minimise acclimation effects and permit recovery between visits, all exercise trials were separated by four days (Pandolf, 1998). For women, all tests were conducted in the same phase of the menstrual cycle, determined by self-reporting using a day counting method (Inoue et al., 2005). All trials were conducted at the same time of day to control for circadian variation. To limit between-investigator error whilst performing measurement techniques, the same member of the research team conducted all trials.

6.3.3. Preliminary testing

During visit 1, participants undertook an incremental exercise test to volitional exhaustion on a cycle ergometer (Monark Exercise AB, Ergomedic 874E, Varberg, Sweden) in hot ambient conditions (37.6 ± 0.4°C and 27.0 ± 5.9% RH) to determine their individual work rates required to elicit $\dot{H}_{prod}$ of ~200, ~250 and ~300 W/m^2 and their peak oxygen consumption ($\dot{V}O_{2peak}$). Participants performed a 5 min warm-up at 80 W, followed by 5 min rest, before commencement of the exercise test. Oxygen consumption ($\dot{V}O_2$) was measured using breath-by-breath expired gas analysis (Jaeger Vyntus CPX, Hoechberg, Germany), with $\dot{V}O_{2peak}$ determined as the highest 30 s mean value, which occurred in the final stage of each participant's test. Criteria for achieving $\dot{V}O_{2peak}$ was: 1) reaching volitional exhaustion; 2) RER > 1.15; 3) final HR within 10 beats/min of age-predicted maximum; and 4) rating of perceiving exertion (RPE) > 19 (6 to 20 Borg scale; Borg, 1982). The test was designed to progressively increase mechanical work rate on the ergometer, in a square-wave manner, to elicit a range of $\dot{H}_{prod}$ values, including those required for exercise in visits 2 and 3. The $\dot{H}_{prod}$ was determined by subtracting the rate of Wk from the rate of metabolic energy expenditure ($\dot{M}$; equation 1).

$$\dot{H}_{prod} = \dot{M} - Wk \text{ [W]} \quad \text{(equation 1)}$$

Where metabolic energy expenditure ($\dot{M}$) was determined using measured $\dot{V}O_2$ and $\dot{V}CO_2$ within the final 1 min of each stage (equation 2):

$$\dot{M} = \dot{V}O_2 \times \frac{\left(\left(\frac{RER-0.7}{0.3}\right)\times 21.13\right)+\left(\left(\frac{1.0-RER}{0.3}\right)\times 19.62\right)}{60} \times 1000 \text{ [W]} \quad \text{(equation 2)}$$

To achieve the necessary $\dot{H}_{prod}$ values, the test was initiated at a mechanical work rate below that which was required to elicit the lowest desired $\dot{H}_{prod}$ (200 W/m^2) and increased by 20 W

every 5 min at a fixed cadence of 80 rpm until exhaustion. The $\dot{H}_{prod}$ (W/m^2) at each stage was estimated based on participant BSA (equation 3 and 4; Cramer & Jay, 2014).

$$\dot{H}_{prod} = \frac{\dot{H}_{prod}}{BSA} \ [W/m^2] \text{ (equation 3)}$$

$$BSA = 0.00718 \times (\text{body mass (kg)}^{0.425}) \times (\text{height (cm)}^{0.725}) \ [m^2] \text{ (equation 4; Dubois \& Dubois equation; DuBois \& DuBois, 1916).}$$

The mechanical work rate required to elicit each target $\dot{H}_{prod}$ (W/m^2) for the exercise trials during visits 2 and 3 (i.e. ~200, ~250 and ~300 W/m^2) was subsequently estimated based on the linear relationship (y = a + b), between $\dot{H}_{prod}$ (W/m^2) and work rate during the incremental test (equation 5).

$$\text{Required work rate} = \frac{\text{Desired } \dot{H}_{prod} \ (W/m^2) - \text{y intercept}}{\text{Slope}} \ [W] \text{ (equation 5)}$$

6.3.4. Inter-day test-retest reliability

6.3.4.1. Pre-exercise instrumentation

Participants were required to arrive euhydrated, as determined by a urine osmolality value < 600 mOsm kg/H_2O (Portable osmometer, Osmocheck, Vitech, Scientific Ltd). If the participant was not deemed to be euhydrated, they were asked to drink 500 ml of plain water and wait 30 min before their urine osmolality was re-measured. Participants wore standardised cycling shorts (94% polyester; 6% elastane), as well as a sports bra for female participants. To measure T_{core}, participants were instructed to insert a flexible rectal thermistor 10 cm past the anal sphincter. Skin thermistors (Grant Instruments Ltd., Cambridge, UK) were attached to four sites on the participant's right side: upper-chest, mid-humerus, mid-calf and mid-thigh to measure mean T_{sk}. Ramanathan's equation (Ramanathan, 1964) was used to calculate mean T_{sk}:

$$T_{sk} = (T_{chest} + T_{arm}) \times 0.3 + (T_{thigh} + T_{calf}) \times 0.2 \ [°C] \text{ (equation 25)}$$

Prior to application of the skin thermistors, the skin was dry-shaved, cleaned with soap and water and allowed to air-dry. Both T_{core} and T_{sk} were continuously recorded using a data logger (SQ2010; Grant Instruments Ltd., Cambridge, UK). Heart rate was continuously recorded throughout each exercise trial (Polar Heart Rate Monitor M400, Warwick, UK). Each

participant's body mass was measured (whilst wearing cycling shorts, a sports bra for females, the HR monitor, the inserted rectal thermistor and the skin thermistors) using a calibrated scale (resolution 50 g; Seca 711, Hamburg, Germany). This was necessary because of the repeated body mass measurements throughout the trial.

6.3.4.2. Exercise trials

Participants initially rested for 30 mins in a seated position in a temperature-controlled room that was regulated to an air temperature of 37.6 ± 0.4°C and a %RH of 27.0 ± 5.9%. Environmental conditions, such as ambient dry-bulb temperature (°C), RH and air velocity (m/s), were continuously monitored (Kestrel 5400 Heat Stress Tracker, Kestrel Meters, Boothwyn, PA, US). An electric fan (SIP 24" Drum Fan, Loughborough, UK) was placed adjacent to the participant during the rest period and diagonally in-front during the exercise periods, providing an airflow of 1 m/s directed at the torso. After 30 min of rest, the participants were seated on the ergometer and performed three exercising periods of 30 min, at the three pre-determined fixed rates of $\dot{H}_{prod}$ (~200, ~250 & ~300 W/m^2). During exercise, oxygen consumption was measured using the same breath-by-breath expired gas analyser (Jaeger Vyntus CPX, Hoechberg, Germany). Participants' body mass was measured, and they were provided with 200 ml of plain water (maintained at room temperature [~20 °C]) to drink between each 30 min period. Rating of perceived exertion was recorded using a 6-20 point Borg scale (Borg, 1982), while thermal comfort (TC) was recorded using a 7-point scale (where -3 = "much too cool", 0 = "comfortable" and 3 = "much too warm"; Bedford, 1936). Thermal sensation (TS) was recorded using a 9-point scale (where -4 = "very cold", 0 = "neutral" and 4 = "very hot"; Zhang et al., 2004). All perceptual data (RPE, TC and TS) were recorded at 5 min intervals during the rest and exercise periods, and upon completion of the trial. Local sweat rate and SGA measurements were taken using the techniques described below.

6.3.4.3. Partitional calorimetry

As detailed in Chapter Three, heat balance parameters such as $\dot{H}_{prod}$ and $\dot{E}_{req}$ (equation 6) were estimated for each 30 min exercise period via partitional calorimetry (Cramer & Jay, 2019). $\dot{H}_{prod}$ was also expressed relative to BSA (DuBois & DuBois, 1916).

$$\dot{E}_{req} = \dot{H}_{prod} - \dot{H}_{dry\ skin} - \dot{H}_{res}\ [W] \quad \text{(equation 6)}$$

6.3.4.4. Local sweat rate measurement

Local sweat rate was determined using the absorbent patch technique (Baker et al., 2018), on the left scapula. Measurements were taken during the final 5 min of each 30 min exercising period. Prior to the trial, the area of interrogation on the skin's surface was shaved and cleaned using water and gauze. A template matching the size of the absorbent patch was pressed to the skin's surface and outlined in indelible ink to identify the area of measurement and ensure consistency of application across the exercise stages and the two inter-day reliability trials. The patch (Medipore + Pad [3M]) was 5 cm x 5.5 cm, with an absorbent capacity of ~7 g. Immediately before patch application, the skin was wiped dry with gauze. The patch was weighed (resolution 0.01 g; Ohaus, Navigator N24120, Nänikon Switzerland) prior to and after the 5 min skin application. Local sweat rate (mg/cm^2/min) was determined as: pre *vs* post change in patch mass in milligrams, divided by the surface area of the patch (5 cm x 5.5 cm) and the duration of application (5 min; equation 26).

$$\text{Local sweat rate} = \frac{\text{pre to post change in patch mass (mg)}}{[5\ (\text{cm}) \times 5.5\ (\text{cm})] \times 5\ (\text{min})}\ [\text{mg/cm}^2/\text{min}] \quad \text{(equation 26)}$$

6.3.4.5. Modified iodine-paper technique

The modified iodine-paper technique (Gagnon et al., 2012) was used to determine SGA on the right scapula. 100% cotton paper (Southworth, Agawam, MA, US) was cut to 9 x 9 cm and further divided into 6 x 6 cm, 3 x 3 cm and 1 x 1 cm sections using a fine-point pencil (Figure 6.1). The paper was placed in an air-tight sealed box, containing iodine in solid form (Sigma-Aldrich, St. Louis, MO). Each 9 x 9 cm piece of cotton paper was suspended from the lid of the container to avoid direct contact with the iodine. The pieces of paper were impregnated with iodine after ~48 h, after which they were removed and placed in sealed bags. Double-sided tape was used to affix the cotton paper to a hard-flat plastic surface to ensure uniform application to the skin. Prior to testing, a 9 x 9 cm template was pressed to the skin's surface at the designated site and outlined in indelible ink to ensure consistency of application across the exercise stages and the inter-day trials. At the end of the rest period and each 30 min exercise period, the skin's surface was blotted dry using a small towel before the iodine-impregnated cotton paper was firmly applied for 5 s. Visually identifiable blue colourations formed on the paper, indicating excreted sweat from active sweat glands during the 5 s application (Figure 6.1). A high-resolution (3024 x 4032) photograph (image) was taken of the paper and subsequently analysed using ImageJ (Rasband, 2011).

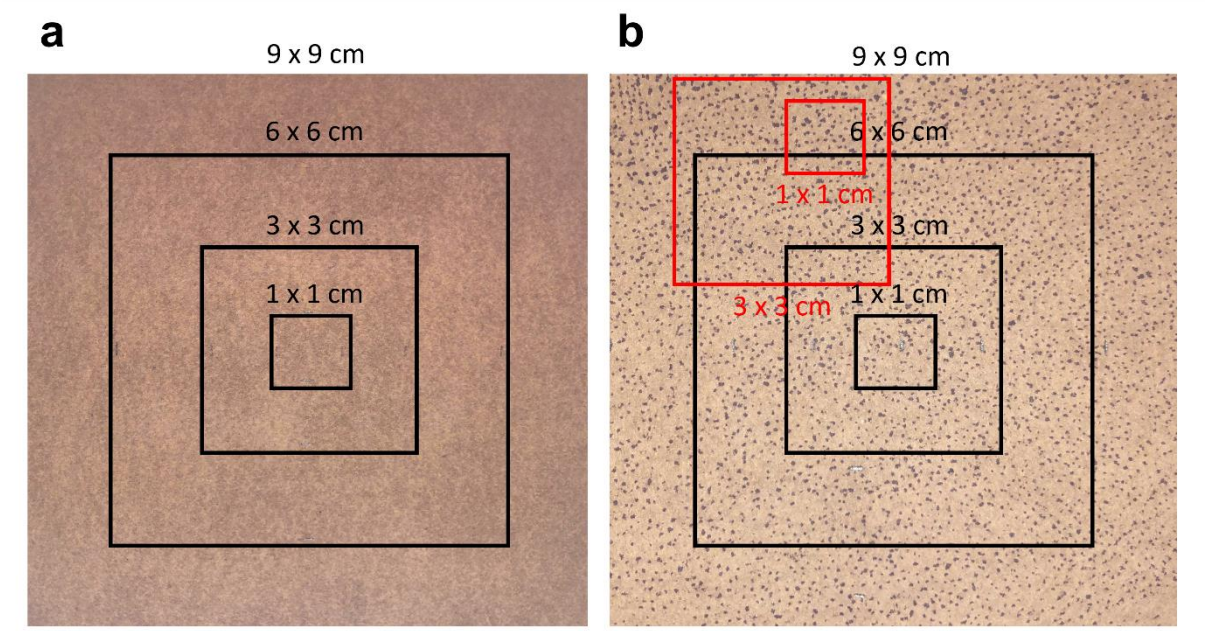


Figure 6.1. (a) Cotton paper divided into pre-determined areas and (b) Iodine impregnated paper displaying sweat gland activation within the pre-determined and optimal (red) areas.

6.3.4.6. Iodine paper image processing and analysis

ImageJ was used to determine i) the number of active sweat glands and ii) the percentage surface area covered with sweat within each pre-determined section of the paper (9 x 9 cm, 6 x 6 cm, 3 x 3 cm, 1 x 1 cm). Further analysis was also performed to determine the optimal area of sweat gland density within 3 x 3 cm and 1 x 1 cm areas within the 9 x 9 cm iodine paper area. This was defined as the area (3 x 3 cm and 1 x 1 cm) with the highest density of recruited glands. All images were taken in a well-lit area to avoid colour contrasts on the paper. For the purposes of replication, please see Appendix B for a detailed guide on ImageJ functions. The number of active sweat glands and the percentage surface area covered in each pre-determined area were later normalised to the maximum value for each individual across all exercise periods. This was to account for inter-individual variation in both the maximal SGA and the point of uncompensability (i.e. individual differences in the balance between evaporative heat transfer [$\dot{E}_{req}$] and the maximum evaporative capacity of the environment [$\dot{E}_{max}$]; Cramer & Jay, 2019).

6.3.5. Statistical analyses

Data were analysed using SPSS (IBM SPSS Statistics for Windows, IBM Corp, Version 24.0. Armonk, New York) and R 4.0.2 (R Core Team, 2018). A 2 (trial 1 and trial 2) x 4 (rest and exercising $\dot{H}_{prod}$ levels) factorial analysis of variance (Two-way repeated measures ANOVA) was conducted to evaluate systematic biases (i.e. differences) in $\dot{H}_{prod}$, $\dot{E}_{req}$, WBSLs, LSR and SGA (gland number, surface area covered, normalised gland number and normalised surface area covered). Identification of interaction effects and Bonferroni-adjusted tests were planned for analysis of any pair-wise systematic biases (Atkinson & Nevill, 1998) in sweat-related measures between trials 1 and 2 at each of the three levels of exercising $\dot{H}_{prod}$. The inter-day reliability was further assessed using the CV (CV% ± 95% confidence limits; Atkinson & Nevill, 1998) on each pairwise comparison. To calculate the CV, the SD of the data was divided by the mean and multiplied by 100 (Atkinson & Nevill, 1998). Repeated measures correlations using the *rmcorr* package in R (v0.4.4; Bakdash & Marusich) were used to establish the relationships between $\dot{E}_{req}$ and $\dot{H}_{prod}$ with WBSL, LSR and SGA (normalised gland number and surface area covered) at each exercise intensity. The repeated measures factor was the participant, as data from each exercise period were pooled, leading to multiple (three) entries from each participant. Confidence intervals were bootstrapped and the CI level was set at 95%. The alpha level (α) for the repeated measures correlations was Bonferroni-adjusted to account for the number of correlations under each hypothesis (n = 14). Data are expressed as means ± SD throughout and a significance level of $p < 0.05$ was accepted across all tests. The thresholds for the magnitudes of effects for correlations were < 0.2, 0.2, 0.5 and 0.8 for trivial, small, moderate and large effects, respectively (Cohen, 1988).

6.4. Results

6.4.1. Whole-body sweat loss and local sweat rate

Participants' mean values for WBSL and LSR during both trials and between-trial reliability (CV%) are presented in Table 6.1. There were no trial main effects or trial x period interaction effects for WBSL (p = .816; p = 0.272, respectively) and LSR (p = .468; p = 0.439, respectively).

Table 6.1. Mean, standard deviation and reliability of whole-body sweat loss and local sweat rate at rest and a range of exercise intensities.

Variable	Trial 1	Trial 2	CV% ± 95% CI
Whole-body sweat loss (g)			
Rest	112 ± 64	108 ± 51	33.5 ± 23.8
200 (W/m^2) $\dot{H}_{prod}$	363 ± 71	333 ± 75	11.0 ± 3.7

250 (W/m^2) $\dot{H}_{prod}$	421 ± 81	437 ± 103	6.4 ± 2.8
300 (W/m^2) $\dot{H}_{prod}$	514 ± 87	523 ± 75	6.0 ± 2.0
All exercise	430 ± 99	429 ± 114	7.8 ± 1.8
Overall	349 ± 167	347 ± 174	14.4 ± 6.8
Local sweat rate (mg/cm^2/min)			
Rest	0.09 ± 0.15	0.07 ± 0.10	77.9 ± 39.1
200 (W/m^2) $\dot{H}_{prod}$	1.06 ± 0.53	0.99 ± 0.37	12.8 ± 4.8
250 (W/m^2) $\dot{H}_{prod}$	1.56 ± 0.60	1.66 ± 0.79	10.1 ± 5.6
300 (W/m^2) $\dot{H}_{prod}$	1.98 ± 0.89	2.16 ± 1.13	18.8 ± 6.1
All exercise	1.52 ± 0.76	1.59 ± 0.93	13.8 ± 3.3
Overall	1.20 ± 0.90	1.20 ± 1.0	30.1 ± 12.8

Note: "All exercise" is the mean of all work rates combined; Trial 1 & 2 data presented as mean ± standard deviation

CV%, coefficient of variation; CI, confidence interval; $\dot{H}_{prod}$, heat production

6.4.2. Sweat gland activation

Participants' mean values for SGA (gland number and surface area covered) during both trials and between trial reliability in the form of CV% are presented in Table 6.2 &Table ***6.3***. There were no trial main effects or trial x period interaction effects for gland number at 9 x 9 cm (p = 0.085; p = 0.488, respectively), 3 x 3 cm (p = 0.210; p = 0.407, respectively), optimal 3 x 3 cm (p = 0.056; p = 0.526, respectively), 1 x 1 cm (p = 0.125; p = 0.217, respectively) and optimal 1 x 1 cm (p = 0.212; p = 0.115, respectively) and surface area covered at 9 x 9 cm (p = 0.785; p = 0.372, respectively), 6 x 6 cm (p = 0.227; p = 0.357, respectively), 3 x 3 cm (p = 0.709; p = 0.407, respectively), optimal 3 x 3 cm (p = 0.133; p = 0.754, respectively), 1 x 1 cm (p = 0.808; p = 0.282, respectively) and optimal 1 x 1 cm (p = 0.571; p = 0.100, respectively). A significant trial main effect was found for gland number at 6 x 6 cm (p = 0.021), but no trial x period interaction effect (p = 0.571).

Table 6.2. Mean, standard deviation and reliability of sweat gland activation (gland number) at rest and a range of exercise intensities.

Variable	Trial 1	Trial 2	CV% ± 95% CI
9 x 9 cm gland number			
Rest	425 ± 435	1,174 ± 1,162	56.1 ± 26.1
200 (W/m^2) $\dot{H}_{prod}$	3,403 ± 1,707	3,864 ± 1,395	20.1 ± 10.8
250 (W/m^2) $\dot{H}_{prod}$	4,888 ± 1,500	5,063 ± 1,833	12.8 ± 4.4
300 (W/m^2) $\dot{H}_{prod}$	6,918 ± 3,228	6,922 ± 2,731	14.7 ± 6.3
All exercise	5,017 ± 2,614	5,236 ± 2,346	15.9 ± 4.5
Overall	3,845 ± 3,032	4,199 ± 2,756	26.2 ± 8.8
6 x 6 cm gland number			
Rest	215 ± 199	691 ± 699	61.9 ± 29.8

200 (W/m^2) $\dot{H}_{prod}$	1,769 ± 906	2,160 ± 817	24.1 ± 11.1
250 (W/m^2) $\dot{H}_{prod}$	2,468 ± 932	2,592 ± 1,001	14.4 ± 7.5
300 (W/m^2) $\dot{H}_{prod}$	3,394 ± 1,988	3,588 ± 1,713	15.2 ± 5.5
All exercise	2,519 ± 1,468	2,757 ± 1,327	18.0 ± 5.0
Overall	1,931 ± 1,623	2,229 ± 1,499	29.2 ± 9.9
3 x 3 cm gland number			
Rest	62 ± 57	200 ± 213	54.1 ± 32
200 (W/m^2) $\dot{H}_{prod}$	502 ± 252	627 ± 264	22.9 ± 11.0
250 (W/m^2) $\dot{H}_{prod}$	690 ± 284	730 ± 290	14.6 ± 7.9
300 (W/m^2) $\dot{H}_{prod}$	913 ± 639	866 ± 361	19.0 ± 11.4
All exercise	695 ± 442	737 ± 313	18.8 ± 5.8
Overall	534 ± 472	600 ± 373	27.8 ± 10
Optimal 3 x 3 cm gland number			
Rest	95 ± 84	237 ± 234	53.7 ± 26.9
200 (W/m^2) $\dot{H}_{prod}$	617 ± 206	629 ± 292	15.9 ± 10.8
250 (W/m^2) $\dot{H}_{prod}$	827 ± 236	899 ± 351	11.5 ± 6.6
300 (W/m^2) $\dot{H}_{prod}$	1,051 ± 666	1,117 ± 657	19.3 ± 10.5
All exercise	825 ± 440	875 ± 485	15.5 ± 5.4
Overall	639 ± 499	712 ± 516	25.2 ± 9.1
1 x 1 cm gland number			
Rest	6 ± 6	22 ± 22	70.0 ± 30.5
200 (W/m^2) $\dot{H}_{prod}$	52 ± 25	68 ± 30	23.3 ± 6.4
250 (W/m^2) $\dot{H}_{prod}$	75 ± 38	79 ± 35	21.1 ± 12.8
300 (W/m^2) $\dot{H}_{prod}$	88 ± 41	90 ± 36	22.2 ± 9.3
All exercise	71 ± 37	78 ± 34	22.2 ± 5.5
Overall	55 ± 43	64 ± 40	34.4 ± 10.5
Optimal 1 x 1 cm gland number			
Rest	14 ± 12	32 ± 31	51.7 ± 24.0
200 (W/m^2) $\dot{H}_{prod}$	75 ± 19	87 ± 34	24.1 ± 9.6
250 (W/m^2) $\dot{H}_{prod}$	110 ± 38	104 ± 32	15.0 ± 7.5
300 (W/m^2) $\dot{H}_{prod}$	116 ± 42	124 ± 34	13.4 ± 8.4
All exercise	100 ± 38	104 ± 35	17.6 ± 5.0
Overall	78 ± 50	86 ± 47	26.3 ± 8.2

Note: "All exercise" is the mean of all work rates combined; Trial 1 & 2 data presented as mean ± standard deviation

CV%, coefficient of variation; CI, confidence interval; $\dot{H}_{prod}$, heat production

Table 6.3. Mean, standard deviation and reliability of sweat gland activation (area covered) at rest and a range of exercise intensities.

Variable	Trial 1	Trial 2	CV% ± 95% CI
9 x 9 cm area covered			
Rest	0.47 ± 0.56%	0.87 ± 0.90%	41.2 ± 22.8
200 (W/m^2) $\dot{H}_{prod}$	2.30 ± 1.85%	2.54 ± 1.44%	26.3 ± 11.1

250 (W/m^2) $\dot{H}_{prod}$	3.92 ± 2.78%	3.89 ± 2.51%	13.8 ± 4.4
300 (W/m^2) $\dot{H}_{prod}$	6.11 ± 3.68%	5.80 ± 2.95%	20.3 ± 7.6
All exercise	4.05 ± 3.17%	4.03 ± 2.66%	20.1 ± 4.9
Overall	3.14 ± 3.16%	3.22 ± 2.71%	25.5 ± 7.2
6 x 6 cm area covered			
Rest	0.44 ± 0.46%	1.00 ± 1.07%	55.7 ± 27.3
200 (W/m^2) $\dot{H}_{prod}$	2.43 ± 1.92%	2.95 ± 1.85%	33.2 ± 11.5
250 (W/m^2) $\dot{H}_{prod}$	3.98 ± 2.78%	3.93 ± 2.63%	14.0 ± 7.1
300 (W/m^2) $\dot{H}_{prod}$	5.95 ± 4.09%	5.81 ± 3.21%	12.6 ± 3.9
All exercise	4.07 ± 3.27%	4.19 ± 2.79%	20.2 ± 5.6
Overall	3.14 ± 3.24%	3.37 ± 2.83%	29.2 ± 9.1
3 x 3 cm area covered			
Rest	0.54 ± 0.55%	1.20 ± 1.28%	52.4 ± 28.1
200 (W/m^2) $\dot{H}_{prod}$	2.71 ± 2.16%	3.43 ± 2.14%	35.4 ± 14.0
250 (W/m^2) $\dot{H}_{prod}$	4.36 ± 3.11%	4.29 ± 2.81%	16.3 ± 10.1
300 (W/m^2) $\dot{H}_{prod}$	6.36 ± 4.86%	5.50 ± 3.02%	23.4 ± 12.5
All exercise	4.42 ± 3.72%	4.38 ± 2.73%	25.4 ± 7.5
Overall	3.43 ± 3.64%	3.56 ± 2.81%	32.3 ± 9.5
Optimal 3 x 3 cm area covered			
Rest	0.86 ± 0.85%	1.51 ± 1.57%	42.6 ± 26.0
200 (W/m^2) $\dot{H}_{prod}$	4.16 ± 3.07%	4.55 ± 2.47%	20.5 ± 13.6
250 (W/m^2) $\dot{H}_{prod}$	5.31 ± 2.39%	5.38 ± 2.58%	16.8 ± 7.1
300 (W/m^2) $\dot{H}_{prod}$	7.90 ± 4.88%	7.82 ± 4.42%	10.5 ± 4.5
All exercise	5.73 ± 3.79%	5.86 ± 3.43%	16.1 ± 5.5
Overall	4.49 ± 3.92%	4.75 ± 3.60%	22.9 ± 8.3
1 x 1 cm area covered			
Rest	0.51 ± 0.42%	1.27 ± 1.34%	62.2 ± 27.2
200 (W/m^2) $\dot{H}_{prod}$	2.77 ± 1.91%	3.65 ± 2.30%	31.9 ± 11.5
250 (W/m^2) $\dot{H}_{prod}$	4.93 ± 3.65%	4.81 ± 2.76%	27.4 ± 12.2
300 (W/m^2) $\dot{H}_{prod}$	6.89 ± 5.75%	5.74 ± 3.14%	30.8 ± 15.8
All exercise	4.80 ± 4.25%	4.70 ± 2.79%	30.0 ± 7.4
Overall	3.71 ± 4.12%	3.83 ± 2.92%	38.2 ± 9.6
Optimal 1 x 1 cm area covered			
Rest	0.74 ± 0.67%	1.74 ± 1.73%	48.6 ± 27.1
200 (W/m^2) $\dot{H}_{prod}$	4.28 ± 2.15%	5.06 ± 2.29%	22.3 ± 12.4
250 (W/m^2) $\dot{H}_{prod}$	6.50 ± 2.67%	6.13 ± 2.62%	20.2 ± 8.6
300 (W/m^2) $\dot{H}_{prod}$	9.41 ± 5.72%	8.89 ± 4.81%	12.5 ± 5.9
All exercise	6.66 ± 4.23%	6.63 ± 3.65%	18.5 ± 5.5
Overall	5.15 ± 4.49%	5.38 ± 3.90%	26.2 ± 8.7

Note: "All exercise" is the mean of all work rates combined; Trial 1 & 2 data presented as mean ± standard deviation

CV%, coefficient of variation; CI, confidence interval; $\dot{H}_{prod}$, heat production

There were also no trial main effects or trial x period interaction effects for normalised gland number at 9 x 9 cm (p = 0.209; p = 0.424, respectively), 3 x 3 cm (p = 0.138; p = 0.459, respectively), optimal 3 x 3 cm (p = 0.442; p = 0.625, respectively), 1 x 1 cm (p = 0.178; p = 0.152, respectively) and optimal 1 x 1 cm (p = 0.484; p = 0.367, respectively) and normalised surface area covered at 9 x 9 cm (p = 0.551; p = 0.456, respectively), 6 x 6 cm (p = 0.123; p = 0.123, respectively), 3 x 3 cm (p = 0.351; p = 0.270, respectively), 1 x 1 cm (p = 0.370; p = 0.325, respectively) and optimal 1 x 1 cm (p = 0.136; p = 0.154, respectively). There was a significant trial main effect, but no trial x period interaction effect for normalised gland number at 6 x 6 cm (p = 0.048; p = 0.362, respectively) and surface area covered at optimal 3 x 3 cm (p = 0.046; p = 0.430, respectively).

6.4.3. Partitional calorimetry

Participants' mean values for absolute $\dot{H}_{prod}$ (W), $\dot{H}_{prod}$ (W/m^2), $\dot{E}_{req}$ (W) and $\dot{E}_{req}$ (W/m^2) during both trials and between trial reliability in the form of CV% are presented in Table 6.4. There were no trial main effects or trial x period interaction effects for $\dot{H}_{prod}$ (p = 0.590; p = 0.112, respectively), $\dot{H}_{prod}$ (W/m^2; p = 0.603; p = 0.126, respectively), $\dot{E}_{req}$ (p = 0.904; p = 0.187, respectively) and $\dot{E}_{req}$ (W/m^2; p = 0.946; p = 0.211, respectively).

Table 6.4. Mean, standard deviation and reliability of partitional calorimetry variables ($\dot{H}_{prod}$ and $\dot{E}_{req}$) at a range of exercise intensities.

Variable	Trial 1	Trial 2	CV% ± 95% CI
$\dot{H}_{prod}$ (W)			
200 (W/m^2) $\dot{H}_{prod}$	377 ± 48	377 ± 48	2.4 ± 1.1
250 (W/m^2) $\dot{H}_{prod}$	474 ± 58	472 ± 52	1.5 ± 0.8
300 (W/m^2) $\dot{H}_{prod}$	564 ± 68	556 ± 64	1.6 ± 0.7
Overall	469 ± 96	466 ± 90	1.8 ± 0.5
$\dot{H}_{prod}$ (W/m^2)			
200 (W/m^2) $\dot{H}_{prod}$	196 ± 19	196 ± 14	2.5 ± 1.1
250 (W/m^2) $\dot{H}_{prod}$	246 ± 20	246 ± 17	1.5 ± 0.8
300 (W/m^2) $\dot{H}_{prod}$	295 ± 22	291 ± 19	1.7 ± 0.7
Overall	244 ± 45	243 ± 42	1.9 ± 0.5
$\dot{E}_{req}$ (W)			
200 (W/m^2) $\dot{H}_{prod}$	373 ± 45	375 ± 41	2.2 ± 0.9
250 (W/m^2) $\dot{H}_{prod}$	463 ± 54	464 ± 50	1.5 ± 0.7
300 (W/m^2) $\dot{H}_{prod}$	544 ± 66	538 ± 62	1.3 ± 0.8
Overall	457 ± 89	457 ± 84	1.7 ± 0.5

$\dot{E}_{req}$ (W/m^2)			
200 (W/m^2) $\dot{H}_{prod}$	194 ± 18	195 ± 13	2.2 ± 0.9
250 (W/m^2) $\dot{H}_{prod}$	241 ± 19	241 ± 17	1.5 ± 0.7
300 (W/m^2) $\dot{H}_{prod}$	285 ± 22	282 ± 19	1.4 ± 0.8
Overall	239 ± 42	238 ± 39	1.7 ± 0.5

Note: Trial 1 & 2 data presented as mean ± standard deviation

CV%, coefficient of variation; CI, confidence interval; $\dot{H}_{prod}$, heat production; $\dot{E}_{req}$, evaporative requirement for heat balance

6.4.4. Correlations

Absolute $\dot{H}_{prod}$ (Figure 6.2), relative $\dot{H}_{prod}$ (Figure 6.3) and absolute $\dot{E}_{req}$ (Figure 6.4) were significantly correlated with WBSL, LSR and SGA (gland number and surface area covered).

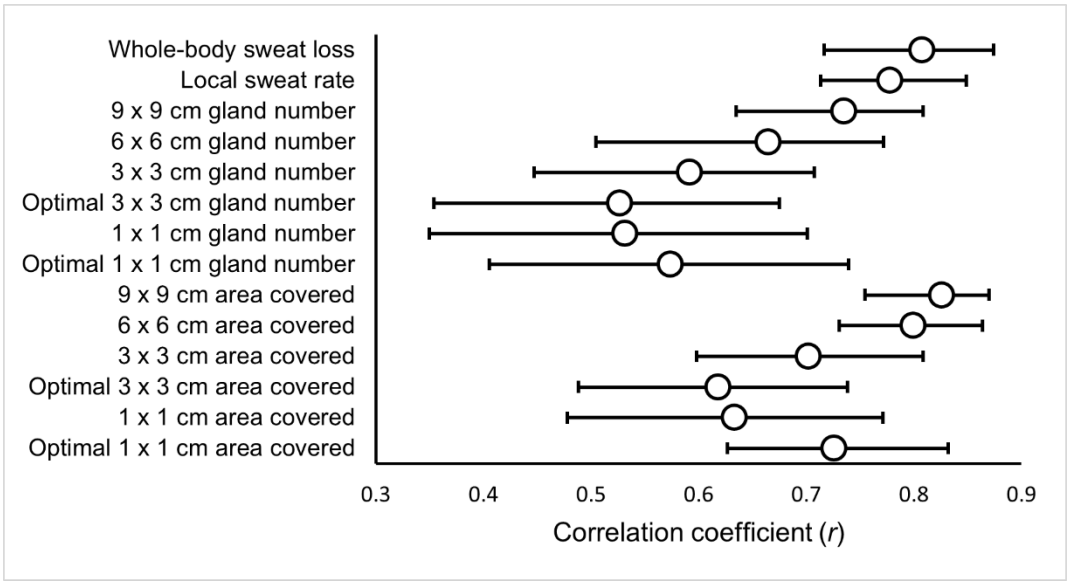


Figure 6.2. The relationship between **absolute** heat production ($\dot{H}_{prod}$) and measures of the sweating response (correlation coefficient ± confidence interval).

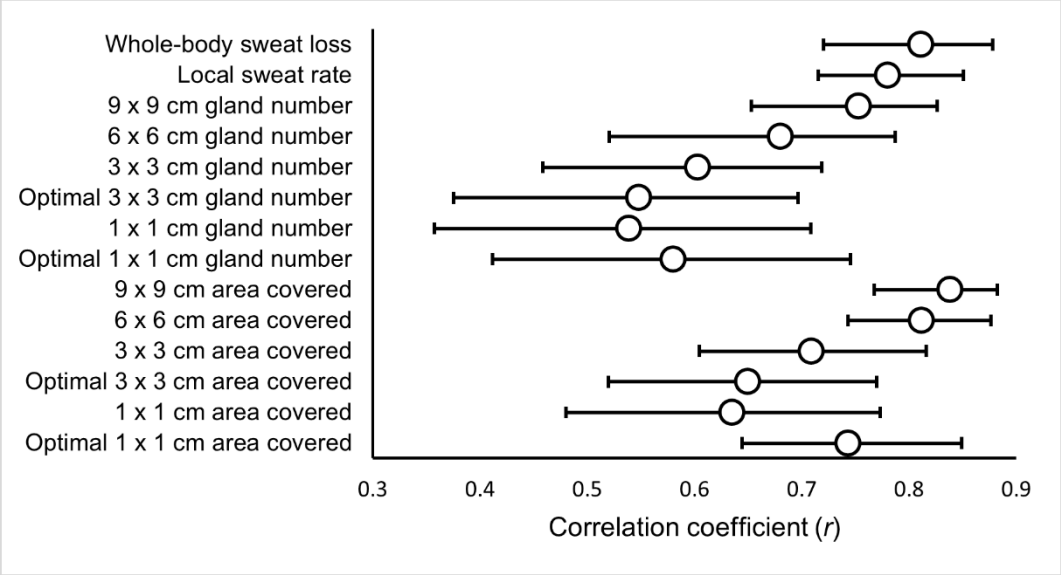


Figure 6.3. The relationship between **relative** heat production ($\dot{H}_{prod}$) and measures of the sweating response (correlation coefficient ± confidence interval).

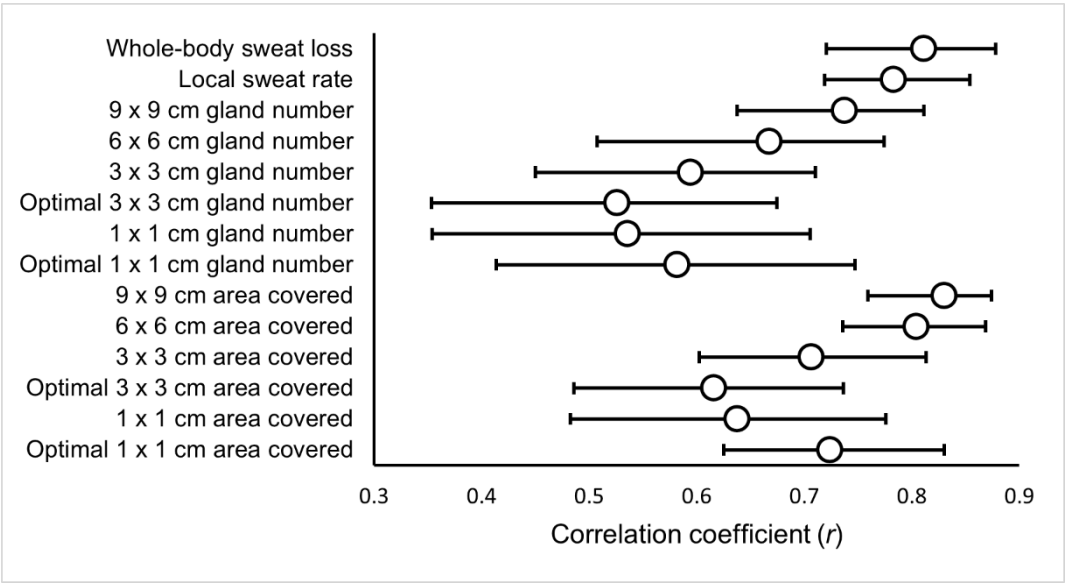


Figure 6.4. The relationship between **absolute** evaporative requirement for heat balance ($\dot{E}_{req}$) and measures of the sweating response (correlation coefficient ± confidence interval).

6.5. Discussion

The current study assessed the inter-day reliability of components of thermal balance and multiple measures of the sweating response at rest and various fixed exercise intensities. The pre-to-post exercise body mass change for measurement of WBSL, the absorbent patch technique for the measurement of LSR (Table 6.1) and the modified iodine-paper technique for the measurement of SGA (both gland counting [Table 6.2] and area covered [Table 6.3]),

were all found to have greater inter-day reliability during the exercising periods compared to rest. Relative and absolute $\dot{H}_{prod}$ and $\dot{E}_{req}$ were found to have very low CVs (Table 6.4), indicating a controlled environment between the two trials. As expected, all measures of the sweating response had strong or moderate correlations with $\dot{H}_{prod}$ (Figure 6.2) and $\dot{E}_{req}$ (Figure 6.4; the drivers of sweat production).

It is important to evaluate the systematic bias (unidirectional error) of a test to understand the potential threats to reliability of a measurement technique (Atkinson & Nevill, 1998). For example, in the current study, it was feasible that participants would demonstrate early-phase adaptation to the heat and exercise stimulus across days, or that the user would improve their technique while using manual methods to assess sweating responses. However, there were no systematic biases detected across all but one of the sweating and calorimetry variables, thus indicating that the trials were conducted in a repeatable manner and the techniques used to characterise the participants' responses were not affected by learning, familiarisation or adaptation effects. Therefore, these techniques, when performed four days apart, can be employed by researchers to control thermal balance and monitor sweating responses among unfamiliarised participants in a laboratory, without concern for systematic adaptations or improvements in the testing procedure. The systematic biases observed for absolute (p = 0.021) and normalised (p = 0.048) sweat gland number using iodine paper at 6 x 6 cm were unanticipated but were largely attributable to two of the participants' measurements. This is likely to be caused by the natural variation in sweat gland recruitment between testing days, which are discussed in later sections of the current discussion in relation to random error.

Determining the pre- *vs* post-trial difference in body mass is the most basic, yet most common method of measuring sweat loss and, thereby, estimating evaporative cooling (Cheuvront & Kenefick, 2017). Despite this, there does not appear to be a study that has investigated the inter-day reliability of WBSL at rest, and multiple fixed exercise intensities. The CVs for WBSL ranged between 6.0 ± 2.0% and 33.5 ± 23.8%. When all exercise work-rates were combined, this was 7.8 ± 1.8%. The CVs decreased (i.e. reliability improved) with increasing exercise intensity, with resting values demonstrating the poorest reliability (33.5 ± 23.8%) and the highest exercise intensity producing the best reliability (300 W/m^2 $\dot{H}_{prod}$; 6.0 ± 2.0%). These results suggest that WBSL has greater reliability at higher work-rates and, consequently, higher sweat rates. This is consistent with the understanding that a greater afferent stimulus for sweating caused by increasing exercise intensities and ambient temperatures elicits a higher frequency and amplitude of sweat gland recruitment (Bini et al., 1980) and, thus, more pronounced effect on sweat production (Kondo et al., 2001). It is likely that the magnitude of stimulus leads to a more sustained effect of sweat production, and therefore more consistent result between trials. On the other hand, the larger CV observed at rest and lower exercise

intensities indicates a greater random error or measurement 'noise', which can be due to technical (instrument dependent variation), biological variation or human error (Hopkins, 2000). The majority of body mass scales have a resolution of ~100 g and therefore smaller body mass changes, as reported at rest, are more difficult to accurately quantify, as they may be just outside the sensitivity of the measurement device. It is important to consider error in relation to 'signal' change; for example, WBSL has been reported to vary by 23% due to heat acclimation (Poirier et al., 2016) and by 13% following supplementation of taurine (Page et al., 2019). These signal changes are greater than the measurement noise (CV%) found during exercise in the current study but are smaller than the CV at rest. Therefore, changes in WBSL should be detectable using this technique during exercising periods but might not be identifiable during rest, owing to the inherent noise of the tests between weeks. It should be mentioned that there are alternative techniques for measuring WBSL with greater accuracy, such as a potter balance, however, these are rarely used due to practicality and limited availability (Cheuvront & Kenefick, 2017). Scales with a resolution of 1 g and accuracy of up to 10 g are also available, though, their high cost can limit their accessibility.

The CVs for LSR, measured using absorbent patches, were 30.1 ± 12.8% overall and 13.8 ± 3.3% for all exercise work rates combined and ranged between 10.1 ± 5.6% and 77.9 ± 39.1%. While the largest CV was also measured during rest (77.9 ± 39.1%), unlike WBSL, these values did not decrease with exercise intensity. The larger CV% found at the highest exercise intensity (Table 6.1) could be attributed to both technical and human sources of error. For example, at higher sweat rates, the absorbent patches' adherence to the skin surface can be compromised, which could affect the amount of sweat absorbed by the patch. In addition, it is well-known that sweating varies across regions of the body, with sweat rates typically higher in the upper-back (Coull et al., 2021). Even within the same region of the body, sweating can be highly variable, depending on specific locations (Havenith et al., 2008). Perhaps less-well recognised is the pulsatile manner of sweat gland innervation and secretion, which varies in amplitude, shape and temporal spacing (Subramanian et al., 2020). Therefore, it is possible that the measurements of localised sweat, such as absorbent patch techniques, could be affected by factors such as the timing of measurement and the consistency of patch application on the upper-back. Additionally, the size of the patches applied, and the resolution of the scales used to weigh them, could further affect the results. Overall, these CV% values suggest that the measure of LSR is slightly less reliable than that of WBSL and could make changes in LSR more difficult to detect, particularly during resting conditions across short time periods. However, the CVs are similar to previous findings, where Baker et al. (2018) reported inter-day reliability of LSR at the scapula during 90 min of cycling at 75 to 80% HR_{max} was 14.5%. The 'noise' reported during exercise in the current study (13.8%) is markedly less than

the expected signal change in LSR on the back with a stimulus such as heat acclimation (~26.7 to 30%; Klous et al., 2020; Ravanelli et al., 2018; Smith & Havenith, 2019).

A poorer reliability at rest was also reflected in the SGA measurements, with resting CV% values ranging from 41.2 ± 22.8% to 70.0 ± 30.5% across the SGA variables and measurement areas. In the exercising periods, however, the CV% values ranged from 15.5 ± 5.4% to 30.0 ± 7.4%, which may be attributed to the same reasons provided for other reliability values of resting sweat measures. These values are slightly higher than the intra-trial CV% values (11 ± 10%) previously reported (Gagnon et al., 2013), suggesting that the reliability of this technique is slightly less between- than within-day, which could be attributed to greater biological variability across the two separate trial days. Sweat gland number and surface area covered at 9 x 9 cm, optimal 3 x 3 cm and optimal 1 x 1 cm were the most reliable (Table 6.2 & Table 6.3). For the optimal 3 x 3 cm and 1 x 1 cm areas this was anticipated, because as part of this new approach, the maximum sweat gland density across all regions of the iodine paper was identified. As discussed herein, sweat gland recruitment patterns are pulsatile in nature and vary across anatomical regions (Coull et al., 2021; Havenith et al., 2008; Subramanian et al., 2020). As such, the pre-designated anatomical regions used in the traditional method are less likely to capture the optimal area of gland activity at any one time-point, leading to increased random error. Searching for the area of optimal gland activity is a more flexible approach, accounting for this variation. The resulting CVs found in this study across exercise intensities supports this. Of course, from a practical perspective, this method is more time-consuming than the traditional measures and researchers should decide whether the incremental improvements (~2%) in reliability warrant the time burden. This decision should be guided by the researcher's analytical goals (Atkinson & Nevill, 1998) but a typical change in SGA as a result of heat acclimation is approximately 27.9% (Ravanelli et al., 2018) and the CV% values reported here for 9 x 9 cm and optimal 3 x 3 cm and 1 x 1 cm (15.5 to 17.6%) would permit detection of these changes. In regard to the reliability of the fixed paper sizes of 9 x 9 cm, 6 x 6 cm, 3 x 3 cm and 1 x 1 cm, this was generally poorer with decreasing paper size for both sweat gland number and area of the paper covered in the exercising periods. Moreover, there is variation in the sweat gland number identified per cm^2 in the larger paper sizes compared to those identified in the smaller ones. This further indicates that SGA is affected by the paper size and, therefore, the results from varying paper sizes are not directly comparable. The explanation for these findings is similar to that provided above, as a larger surface area will more likely include the optimal area of gland activity and account for variability across the body region, which may not be included in smaller paper sizes.

For the first time, measurement of the surface area covered, rather than activated sweat gland number was investigated. This could be more reliable than gland number on the basis that co-

joining of neighbouring glands could be incorrectly measured as single glands using the counting method, leading to measurement error. It is theorised this would most likely be the case at higher sweat rates (i.e. $\dot{H}_{prod}$ of 300 W/m^2), where dots on the paper would be larger and output per gland would be taken into account, as well as number of activated glands. However, findings were equivocal with some paper sizes more or less reliable for gland number *vs* surface area covered. Nevertheless, these measures performed very similarly in regard to reliability, with the surface area covered marginally less reliable than sweat gland number by approximately ~1.9% across the paper sizes (Table 6.2 & Table 6.3). Therefore, it has been concluded that either surface area covered, or the counting method could be used but recognise that the counting method may be preferable for researchers intending to combine sweat gland recruitment with LSRs to determine output per gland (Ravanelli et al., 2018).

Indeed, it has been shown that progressive recruitment of sweat glands occurs during early exercise in the heat, but at latter stages, LSR is reliant upon sweat production per activated gland (Kondo et al., 2001). This might also support the current findings, where the 250 and 300 W/m^2 $\dot{H}_{prod}$ exercise intensity produced the most reliable sweat gland measurements. Owing to the study design, the highest work-rates occurred during later stages of exercise and would naturally have recruited most or all of the available sweat glands, leading to greater consistency in the results. Nevertheless, the noise associated with this technique should be considered by potential users and the manual nature of the paper application, particularly during exercise, will inevitably lead to some human error and some unreliability, which might prevent detection of 'small' changes in sweat gland activity.

This study appears to be the first to directly report on the inter-day reliability of selected components of thermal balance using partitional calorimetry; namely, absolute and relative $\dot{H}_{prod}$ and $\dot{E}_{req}$. Both absolute $\dot{H}_{prod}$ (CV% = 1.8 ± 0.5) and $\dot{E}_{req}$ (CV% = 1.7 ± 0.5) and relative $\dot{H}_{prod}$ (CV% = 1.9 ± 0.5) and $\dot{E}_{req}$ (CV% = 1.7 ± 0.5) were reliable across all exercise intensities. Given that $\dot{H}_{prod}$ and $\dot{E}_{req}$ are determined by a combination of measured variables, such as $\dot{V}O_2$ (CV% = 1.7 ± 0.5), Wk and ambient conditions (°C [CV% = 0.2 ± 0.1] and %RH [CV% = 4.3 ± 1.4]), which are commonly used laboratory measures, these results were anticipated. It was necessary to establish the reliability of these variables because of their reported relationship with selected sweat measurements (Gagnon et al., 2013; Ravanelli et al., 2020). Indeed, as drivers of thermal sweating, consistent control of these thermal balance components is necessary for reliable measurement of sweat responses.

As anticipated, WBSL showed strong significant positive correlations with both absolute $\dot{H}_{prod}$ and $\dot{E}_{req}$ (r_{rm} = 0.81; r_{rm} = 0.81, respectively). There were also moderate to strong, significant

positive correlations with $\dot{H}_{prod}$ and $\dot{E}_{req}$ for LSR (r_{rm} = 0.78; r_{rm} = 0.78, respectively). This is consistent with previous reports, where WBSR (adjusted R^2 = 0.64; adjusted R^2 = 0.78, respectively; Gagnon et al., 2013; Hospers et al., 2020) and LSR (Ravanelli et al., 2020), as measured by the ventilated capsule technique have been associated with absolute $\dot{H}_{prod}$ and $\dot{E}_{req}$. Additionally, LSR at the arm (r = 0.62; p = 0.03; r = 0.38; p = 0.23) and forehead (r = 0.56; p = 0.06; r = 0.31; p = 0.33), determined using absorbent patches, has been moderately correlated with $\dot{E}_{req}$ and $\dot{H}_{prod}$ respectively (Bain et al., 2011). To expand upon these findings, the relationship between other sweat measures, such as SGA, and $\dot{H}_{prod}$ and $\dot{E}_{req}$ were evaluated using an appropriate statistical technique that accounts for repeated observations across the three exercise intensities. Overall, SGA (both gland number and surface area covered), normalised to maximum values, were related to absolute $\dot{H}_{prod}$ and $\dot{E}_{req,}$ with surface area covered at 9 x 9 cm (r_{rm} = 0.83; r_{rm} = 0.83, respectively) and 6 x 6 cm (r_{rm} = 0.80; r_{rm} = 0.80, respectively) demonstrating the strongest relationships. All other SGA variables demonstrated *moderate* correlations (Figure 6.2 &Figure ***6.4***). The larger iodine paper sizes showed stronger relationships with $\dot{H}_{prod}$ and $\dot{E}_{req}$, across both methods of establishing SGA. In addition, the relationships between $\dot{H}_{prod}$ and $\dot{E}_{req}$ were consistently greater for surface area covered compared to number of active sweat glands counted. Collectively, these results suggest that larger paper sizes and using the surface area covered method were more consistently associated to the pre-established drivers of thermal sweating. However, all SGA variables related positively with $\dot{H}_{prod}$ and $\dot{E}_{req,}$ and, therefore thermal stimuli, and are consequently valid. The reliability of the method should also be considered when deciding upon the method used to establish SGA.

6.5.1. Limitations, future directions and recommendations

The scales used in this study to measure changes in body mass (for the estimation of WBSL) and the absorbent patches (for estimation of LSR) had resolutions of 50 g and 0.01 g, respectively. Scales with greater resolution may provide slightly different results, especially at rest, as discrete changes in WBSL < 50 g and LSR < 0.01 g would not have been detected in the current study. However, during exercise the results were sufficiently reliable within the limits of the equipment used. The equipment used here can be found in most standard exercise physiology laboratories, which supports the generalisability of the current findings. However, reliability is dependent on the techniques used, which can be affected by various sources of error, caused by factors such as the use of different equipment across laboratories, or the skill and application of different investigators. These factors should be considered when utilising the current data for future purposes.

Both researchers and practitioners, across all applied scientific fields, can use the data reported in the current reliability study to set analytical goals (Atkinson & Nevill, 1998) for their research. For example, future users might determine the minimum changes in sweat rate or gland activation as a result of training that are necessary to be deemed beyond the margin of error. Changes in WBSL, LSR and SGA would need to be greater than 7.8%, 13.8% and 15.5 to 17.6%, respectively to be considered genuine, based on the findings herein. A change greater than the noise of the techniques established in this study should be considered meaningful. Thus, these techniques can be used in any study or intervention aiming to determine changes in sweating using these techniques. Furthermore, researchers in the laboratory or field can use the data reported here to determine the comparability, or perhaps acceptability, of their own techniques. Finally, the CV% values reported herein can also be used to determine appropriate sample sizes for future studies. When planning studies, the simple use of a nomogram (Batterham & Atkinson, 2005) could be used in conjunction with the current data when an *a-priori* notion of the change in an outcome measure has been established. For example, based on the CV% values reported here, a 10% change in WBSL could be detected with a sample size of approximately 15.

6.5.2. Conclusion

As was anticipated, all sweating response variables were positively related with $\dot{H}_{prod}$ and $\dot{E}_{req}$. Absolute and relative $\dot{H}_{prod}$ and $\dot{E}_{req}$ demonstrated inter-day reliability, adequate to control the thermal sweating response. The pre- *vs* post-exercise body mass change for measurement of WBSL, the absorbent patch technique for the measurement of LSR and the various methods of establishing SGA were all also found to have inter-day reliability during the exercising periods, sufficient to detect changes in thermal sweating that might occur following an intervention, such as heat acclimation or dietary manipulation. This was not the case at rest, however, and therefore these methods would be unlikely to be able to detect any changes to the resting sweating response. The modified iodine-paper technique was marginally more reliable at 9 x 9 cm and when the 3 x 3 cm and 1 x 1 cm area of optimal sweat gland density was determined, particularly when measuring the sweat gland number, as opposed to the surface area covered. The larger paper sizes (9 x 9 cm and 6 x 6 cm) had the strongest relationships with $\dot{H}_{prod}$ and $\dot{E}_{req}$, especially when SGA was measured using surface area covered. It is, therefore, recommend that to establish SGA at the upper-back, 9 x 9 cm paper sizes are used, with the option of identifying the 3 x 3 cm or 1 x 1 cm optimal areas if deemed necessary. The method of analysis applied (i.e. gland counting or surface area covered) should be chosen based on the research aim.

7. Chapter Seven – The effect of eight-days oral taurine supplementation on thermoregulation during low-intensity exercise at fixed heat production in hot conditions of incremental humidity

The study that comprises Chapter Seven has been published in the European Journal of Applied Physiology. Chapter Seven is identical to the published version, with the only adaptations due to required formatting alterations, changes to the study's power analysis and the addition of expired gas analysis data.

Reference: Peel, J. S., McNarry, M. A., Heffernan, S. M., Nevola, V. R., Kilduff, L. P., Coates, K., Dudley, E., & Waldron, M. (2024). The effect of eights-days oral taurine supplementation on thermoregulation during low-intensity exercise at fixed heat production in hot conditions of incremental humidity: a double-blind, placebo-controlled trial. *European Journal of Applied Physiology,* 1-16.

7.1. Abstract

Background Exercise in hot and/or humid environmental conditions can cause positive heat storage if thermal equilibrium is not maintained through sufficient dry or latent heat dissipation. This may lead to uncompensable stress, denoted by inexorable increases in T_{core}. Thermal sweating and the efficiency of evaporative heat transfer can be enhanced through interventions, such as endurance training and heat acclimation, delaying this upward inflection in T_{core}. Similarly, taurine supplementation has increased sweat rate and hastened the onset of sweating during exhaustive exercise in the heat, but its potential role in thermoregulation has not been thoroughly evaluated.

Objectives To determine the effect of oral taurine supplementation on sweating and T_{core} responses, including the transition from compensable to uncompensable heat stress, and calorimetric heat transfer components during prolonged low-intensity exercise of a fixed heat production (~200 W/m^2) in hot ambient temperatures of 37.5°C, at both fixed and incremental vapour pressure.

Methods Fifteen, healthy, non-heat acclimated female (n = 3) and male (n = 12) participants (27 ± 5 years, 78 ± 9 kg, $\dot{V}O_{2max}$ 50.3 ± 7.8 mL/kg/min), completed a treadmill walking protocol (~200 W/m^2 $\dot{H}_{prod}$) in the heat (37.5 ± 0.1°C) at fixed- (16 mmHg) and ramped-humidity (Δ1.5 mmHg/5 min) following eight days of oral taurine supplementation (50 mg/kg/bm) or placebo,

in a double-blind, randomised, cross-over design. Participants were assessed for WBSL, LSR, SGA, T_{core}, breakpoint of compensability (P_{crit}) and calorimetric heat transfer components. Plasma volume and plasma taurine concentrations were established through pre- and post-trial blood samples.

Results Taurine supplementation increased WBSL by 26.6% (662 ± 224 *vs* 523 ± 130 mL) and 5.1% (797 ± 159 *vs* 758 ± 161 mL; $p = 0.035$), LSR by 15.5% (1.38 ± 0.55 *vs* 1.19 ± 0.42 mg/cm^2/min) and 7.8% (3.2 ± 1.6 *vs* 3 ± 1.5 mg/cm^2/min; $p = 0.013$), SGA (1 x 1 cm) by 32.2% (90 ± 33 *vs* 68 ± 28 gland no.) and 29.9% (108 ± 34 *vs* 83 ± 23 gland no.; $p < 0.001$) and SGA (3 x 3 cm) by 22.1% (666 ± 279 *vs* 545 ± 244 gland no.) and 17.1% (751 ± 243 *vs* 642 ± 213 gland no.; $p = 0.015$) during the fixed- and ramped-humidity exercise periods, respectively. Evaporative heat loss was enhanced by 27% (595 ± 201 *vs* 470 ± 116 W; $p = 0.010$), heat-storage reduced by 72% (30 ± 104 *vs* 108 ± 73 W; $p = 0.024$) and P_{crit} was greater in taurine *vs* placebo (25.0 mmHg *vs* 21.7 mmHg; $p = 0.002$).Taurine supplementation increased plasma taurine concentrations compared to placebo (254 ± 198 μM *vs* 82 ± 59 μM, respectively).

Conclusion Taurine supplementation increased sweating responses during fixed $\dot{H}_{prod}$ in hot conditions, prior to substantial heat strain and before the breakpoint of compensability, demonstrating improved thermoregulatory capacity. The enhanced evaporative cooling and reduced heat storage delayed the subsequent upward inflection in T_{core} – represented by a greater P_{crit} – and, therefore, taurine offers a potential dietary supplementation strategy to support thermoregulation.

7.2. Introduction

Exercise increases $\dot{H}_{prod}$, with dry (conduction, convection and radiation) and evaporative heat exchange providing potential avenues of heat dissipation (Gagge & Gonzalez, 1996). In hot environmental conditions, evaporative heat transfer (latent heat transfer; $\dot{E}_{skin}$) is the main and most modifiable avenue of heat dissipation, which occurs secondary to sweating when ambient vapour pressure permits (Wenger, 1972). Indeed, both $\dot{H}_{prod}$ and $\dot{E}_{req}$ drive the thermal sweating response (Cramer & Jay, 2014, 2016; Gagnon et al., 2013; Chapter Six). Consequently, when exercising or performing occupational work in the heat, eccrine sweat production is an important physiological mechanism for the maintenance of thermal equilibrium, as it allows for evaporative cooling to offset $\dot{H}_{prod}$ (Marino et al., 2000; Sawka & Young, 2006). Thus, manipulation of factors affecting calorimetric components, such as $\dot{H}_{prod}$ or any heat loss avenue, will affect thermal balance.

Evaporation of sweat off the skin's surface, and therefore the latent heat of vaporisation, is determined by both the efficiency of sweating and the ambient vapour pressure (determined from the relationship between air temperature and RH (Gagge & Gonzalez, 1996; Parsons, 2007). Dry, hot environmental conditions allow for a greater capacity to evaporatively cool compared to those with high humidity (Che Muhamed et al., 2016). This is due to the larger vapour pressure gradient between the ambient air and skin's surface, which permits a superior maximal evaporative heat transfer capacity ($\dot{E}_{max}$; Cramer & Jay, 2019). Consequently, prolonged exercise in hot and humid environmental conditions causes thermal strain (positive heat storage), due to the reduced capacity for evaporative cooling. Indeed, without sufficient dry and evaporative heat transfer, positive heat storage will ensue, forcing a transition from a compensable to an uncompensable state, denoted by inexorable increases in T_{core} (Cramer & Jay, 2016; Marino et al., 2000). Thermal sweating and the efficiency of evaporative heat transfer can be acutely enhanced through interventions, such as endurance training and heat acclimation (Ravanelli et al., 2018). This training- or acclimation-induced augmentation of the sweating response was reported to delay the upward inflection in T_{core} associated with a transition to thermal uncompensability using an 'inflection protocol', whereby ambient vapour pressure is manipulated at fixed ambient dry-bulb temperature and heat production (Ravanelli et al., 2018). Thus, manipulation of the environmental conditions can be useful in determining capacity to thermoregulate and changes that might occur following interventions, such as dietary supplementation.

Taurine, a sulphur containing amino acid, can be supplemented orally and has been shown to enhance endurance exercise performance in thermoneutral conditions (Balshaw et al., 2013; Waldron et al., 2019; Waldron et al., 2018a; Zhang et al., 2004). These ergogenic effects mimic the magnitude of endurance training responses to heat acclimation (Waldron et al., 2021) and appear to be related to sarcoplasmic reticulum Ca^{+} handling (Dutka et al., 2014; Hamilton et al., 2006), anti-oxidative effects (Hansen et al., 2006; Hansen et al., 2010; Jong et al., 2021; Schaffer et al., 2022; Zhang et al., 2004) and/or alterations in substrate utilisation, favouring greater relative fat oxidation (Rutherford et al., 2010; Simmonds et al., 2022), which is a feature of the endurance trained phenotype (Lima-Silva et al. 2010). Further, taurine has many other biological roles that could be advantageous to exercise performance in the heat, including cellular osmoregulation (Cuisinier et al., 2002) and vaso-active properties (Maia et al., 2014; Sun et al., 2016; Ulusoy et al., 2017). The osmoregulatory capacity of taurine might be exaggerated following oral supplementation, with higher plasma taurine concentrations increasing osmotic pressure in both central and peripheral sites, thereby acutely drawing fluid into the vascular space and theoretically sustaining, or perhaps expanding, plasma volume. However, it should be noted that taurine has a weaker relationship with plasma volume

compared to other osmolytes, such as sodium and chloride (Cuisinier et al., 2002) and the effect of exogenous supplementation on plasma volume in heat stressed, exercising humans has not yet been established. Nonetheless, various beneficial physiological effects of taurine supplementation have been demonstrated in hot conditions, as described below. Indeed, the performance enhancing effect of taurine supplementation was heightened when administered during exercise in the heat, prolonging time to exhaustion by 10% (Page et al., 2019). Here, taurine increased the rate (~12.7%) and hastened the onset of sweating during exhaustive exercise in the heat, alongside substantial reductions in T_{core} (end T_{core} of 38.1°C *vs* 38.5°C), demonstrating its potential role in thermoregulation. The early changes in the sweating response were considered to be indicative of a centrally-mediated alteration in thermoregulatory set-points, which might relate to its role as a neuromodulator (Hussy et al., 2000; Jia et al., 2008). However, despite this early promising research, it remains necessary to more comprehensively evaluate the potential thermoregulatory role of taurine supplementation in a controlled experimental setting, where sufficient control of calorimetric components is permitted (i.e. $\dot{H}_{prod}$ and $\dot{E}_{req}$). Furthermore, the effect of taurine on the sweating response and subsequent thermoregulation across more prolonged exercise periods is unknown.

The aim of the current study was to determine the effect of an eight-day taurine supplementation period on T_{core} and sweating responses (WBSL, LSR and SGA), calorimetric heat transfers components ($\dot{E}_{skin}$ and heat storage), delta plasma volume, and plasma taurine concentrations during prolonged low-intensity exercise of a fixed $\dot{H}_{prod}$ in the heat at both fixed and increasing vapour pressure. It was hypothesised that taurine supplementation would: i) induce greater sweating responses across the exercise protocol; ii) delay the increase in T_{core} during the period of increasing vapour pressure (transition to an uncompensable environment); iii) increase plasma volume and; iv) result in greater evaporative heat transfer and reduced heat storage, as modelled by partitional calorimetry.

7.3. Methods

7.3.1. Participants

Fifteen non-heat acclimated, healthy females (*n* = 3) and males (*n* = 12) volunteered to take part in the study (27 ± 5 years, 179 ± 8 cm, 78 ± 9 kg, maximal oxygen uptake ($\dot{V}O_{2max}$) 50.3 ± 7.8 mL/kg/min). Based on the effect sizes (Cohen's *d* = 0.72) reported using taurine to increase the sweating response in the heat (Page et al., 2019), G∗Power (Version 3.0.10; Universität Düsseldorf, Germany) was used to calculate an appropriate *a-priori* sample size of 15 to identify significant differences between groups. As part of the health screening

questionnaire, participants were asked if they had been exposed to hot ambient temperatures in the previous two months, sufficient to induce heat adaptation and were excluded if so. Participants were asked to refrain from alcohol and caffeine consumption for 24 h and to avoid strenuous exercise and follow a consistent diet for 48 h prior to testing. They were provided with a food diary to record their food intake and asked to replicate this prior to all subsequent visits. Use of any performance enhancing or dietary supplements, such as caffeine, was prohibited for the duration of the study. This was verified in the pre-trial screen, along with the opportunity for participants to report any adverse health effects. All female participants were on hormonal oral contraceptive pills. Written informed consent was obtained from all participants. Institutional ethics approval (JP_30-10-20b; Appendix A) was provided and the study was conducted in accordance with the 2013 Declaration of Helsinki, except for pre-registration on a publicly accessible database.

7.3.2. Design

This study adopted a double-blind, randomised, placebo-controlled, cross-over design. Participants reported to the laboratory on five separate occasions; once for pre-screening and familiarisation (visit 1), twice to complete a walking incremental test to establish the work rate-$\dot{V}O_2$ relationship and $\dot{V}O_{2max}$ (visits 2 & 4) and twice for the experimental trials, in which they completed a fixed- and ramped-humidity treadmill walking protocol, following eight-days of supplementation with either 50 mg/kg/bm of taurine or 30 mg/kg/bm of maltodextrin (placebo; visits 3 & 5; Figure 7.1). All testing sessions took place in an environmental chamber set to 37.5 ± 0.1°C and 34.2 ± 1.4% RH. The break period of 7-days between conditions was selected to permit complete recovery from the protocols and time to consume the cross-over supplementation. Taurine has a ratio of clearance/bioavailability of ~21 h and, therefore, this was considered a sufficient washout period (Ghandforoush-Sattari et al., 2010). All trials were conducted at approximately the same time of day to control for circadian rhythm variation. Randomisation was performed manually via coin toss by an independent person.

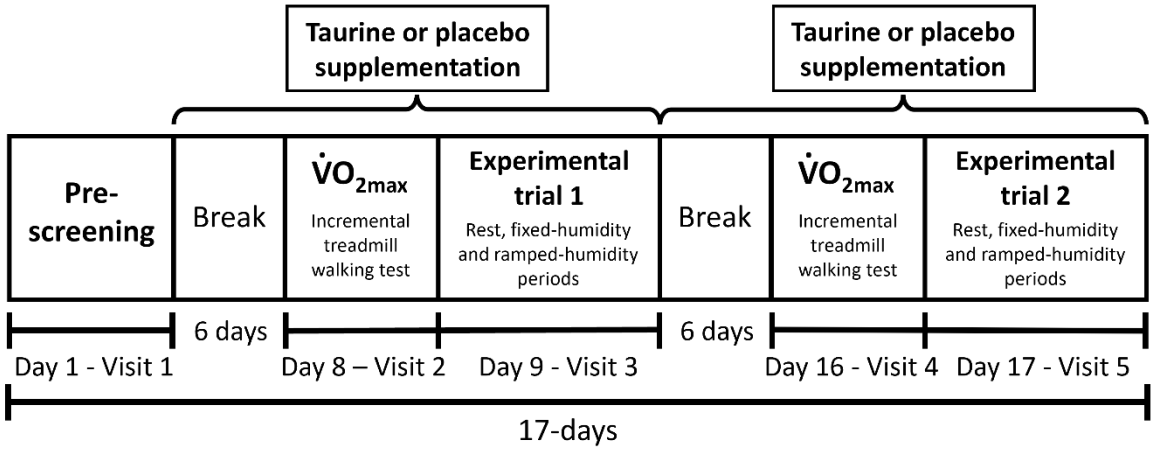


Figure 7.1. Schematic of the study timeline. $\dot{V}O_{2max}$ = maximal oxygen uptake

7.3.3. Incremental walking test

During visits 2 and 4, participants completed an incremental treadmill walking test to volitional exhaustion on a calibrated treadmill (h/p/cosmos, Am Sportplatz 8, Germany) in hot conditions (37.5 ± 0.1°C and 34.2 ± 1.5% RH) to determine their $\dot{V}O_{2max}$ and individual work rates required to elicit $\dot{H}_{prod}$ of ~200 W/m^2. The test began at 2 km/h (0.56 m/s) and increased by 1 km/h (0.28 m/s) every 3 min stage with corresponding gradients of 0% in the first stage, 5% in the second stage, 10% in the third stage, and 2% additional increases thereafter. The incremental test was conducted to volitional exhaustion. Pulmonary $\dot{V}O_2$ was measured using breath-by-breath expired gas analysis (Jaeger Vyntus CPX, Hoechberg, Germany), with $\dot{V}O_{2max}$ determined as the highest 30 s mean value, which occurred in the final stage of each participants' test. Time to exhaustion was determined as the time at which volitional exhaustion occurred. The test was designed to progressively increase mechanical work rate, in a square-wave manner, to elicit a range of $\dot{H}_{prod}$ values, including that required for the treadmill walking protocol in visits 3 and 5 (200 W/m^2). Each participant's $\dot{H}_{prod}$ for the experimental trials were determined by subtracting the rate of mechanical work (Wk) from the rate of metabolic energy expenditure ($\dot{M}$; equation 1).

$$\dot{H}_{prod} = \dot{M} - \dot{W}k\ [W] \text{ (equation 1)}$$

Where: metabolic energy expenditure ($\dot{M}$) was determined using measured $\dot{V}O_2$ and RER in the final 1 min of each incremental stage (equation 2):

$$\dot{M} = \dot{V}O_2 \times \frac{\left(\left(\frac{RER-0.7}{0.3}\right)\times 21.13\right)+\left(\left(\frac{1.0-RER}{0.3}\right)\times 19.62\right)}{60} \times 1000\ [W] \text{ (equation 2)}$$

To achieve the necessary $\dot{H}_{prod}$ value, the test was initiated at a mechanical work rate below that required to elicit the desired $\dot{H}_{prod}$ (200 W/m^2) and increased until exhaustion. The $\dot{H}_{prod}$ (W/m^2) at each stage was determined based on participant BSA (equation 3 and 4; Cramer & Jay, 2014).

$$\dot{H}_{prod} = \frac{\dot{H}_{prod}}{BSA} \ [W/m^2] \text{ (equation 3)}$$

$$BSA = 0.00718 \times (\text{body mass (kg)}^{0.425}) \times (\text{height (cm)}^{0.725}) \ [m^2] \text{ (equation 4; DuBois \& DuBois, 1916).}$$

The mechanical work rate required to elicit each target $\dot{H}_{prod}$ (W/m^2) for the exercise trials during visits 3 and 5 (i.e. ~200 W/m^2) was subsequently determined based on the linear relationship (y = mx + b), between $\dot{H}_{prod}$ (W/m^2) and work rate during the incremental test (equation 5). This equated to ~43.1 ± 6.2 W and ~34.7 ± 5.5% of $\dot{V}O_{2max}$ in the taurine condition and ~42.1 ± 6.2 W and ~34.9 ± 5.6% of $\dot{V}O_{2max}$ in the placebo condition.

$$\text{Required work rate} = \frac{\text{Desired } \dot{H}_{prod} \ (W/m^2) - \text{y intercept}}{\text{Slope}} \ [W] \text{ (equation 5)}$$

7.3.4. Experimental trials

7.3.4.1. Pre-trial instrumentation

Participants were required to arrive euhydrated, as determined by a urine osmolality value < 600 mOsm kg/H_2O (Portable osmometer, Osmocheck, Vitech, Scientific Ltd). If the reading was > 600 mOsm kg/H_2O (the threshold for hypohydration) the participant was asked to drink 500 mL of plain water and wait 30 min. Urine osmolality was then re-determined and if the participant was deemed euhydrated, testing commenced. Participants wore running shorts (90% polyester, 10% elastane), as well as a sports bra for female participants. To measure T_{core}, participants were instructed to insert a flexible rectal thermistor 10 cm past the anal sphincter (Walters Medical, W0001B, England).

7.3.4.2. Trials (rest, fixed-humidity and ramped-humidity)

Participants initially rested for 30 min in a seated position within the environmental chamber, which was regulated to an ambient dry-bulb temperature (T_{db}) of 37.5 ± 0.1°C, RH of 34.2 ± 1.4% and vapour pressure of 16 mmHg. Environmental conditions, such as ambient T_{db}, RH and air velocity (m/s), were continuously monitored approximately 120 cm from the exercising participant (Kestrel 5400 Heat Stress Tracker, Kestrel Meters, Boothwyn, PA, US). A large

electric fan (SIP 24" Drum Fan, Loughborough, UK) was placed in front of the participant during the rest and exercise periods, providing an airflow of 2.0 ± 0.2 m/s, directed at the torso. During the rest period, skin thermistors (Grant Instruments Ltd., Cambridge, UK) were attached to four sites on the participant's left side: upper-chest, mid-humerus, mid-calf and mid-thigh to measure weighted mean T_{sk}. Prior to application of the skin thermistors, the skin was dry-shaved. Both T_{core} and T_{sk} were recorded using a data logger, continuously sampling every 5 s (SQ2010; Grant Instruments Ltd., Cambridge, UK). Ramanathan's equation (Ramanathan, 1964) was used to calculate mean T_{sk}:

$$T_{sk} = (T_{chest} + T_{arm}) \times 0.3 + (T_{thigh} + T_{calf}) \times 0.2 \ [°C] \text{ (equation 25)}$$

After 30 min of rest in the chamber, the participants began walking on the treadmill at an individual-specific speed and gradient intended to elicit a pre-determined fixed $\dot{H}_{prod}$ (~200 W/m^2). After 45 min of exercise (fixed-humidity exercise period), the ambient vapour pressure (mmHg) inside the environmental chamber increased by 1.5 mmHg every 5 min for an additional 60 min (ramped-humidity exercise period). The point at which an upward inflection in T_{core} was observed was identified as the critical ambient vapour pressure (P_{crit}), theoretically indicating the transition from a compensable to an uncompensable state (Kenney & Zeman, 2002; Ravanelli et al., 2018; Figure 7.2 & Figure 7.3). The inflection in T_{core} at the breakpoint of compensability (P_{crit}) was determined using segmental linear regression of the T_{core} – ambient vapour pressure relationship, which was averaged to 1 min values during the ramped-humidity exercise period (Graphpad Prism, version 5.01, La Jolla, CA). Participants were provided with 200 mL of plain water (maintained at room temperature [~20°C]) after the rest period and before exercise, and 400 mL between the 45 min fixed-humidity and 60 min ramped-humidity exercise periods. Fluid intake was later accounted for when determining changes in body mass losses at selected trial stages.

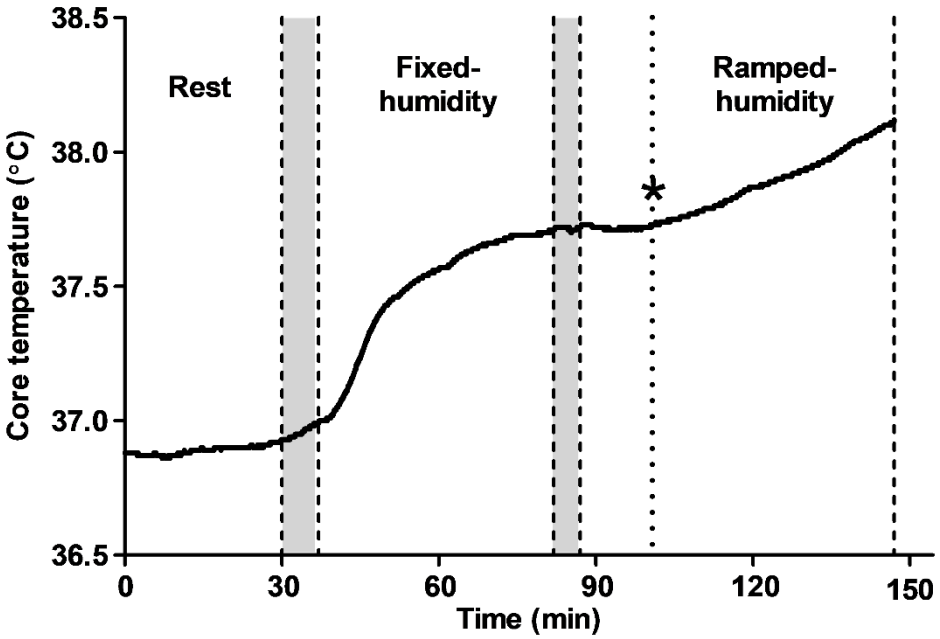


Figure 7.2. Example core temperature time series of a representative participant during the experimental trial. *Point of inflection, Grey bars = transition periods.

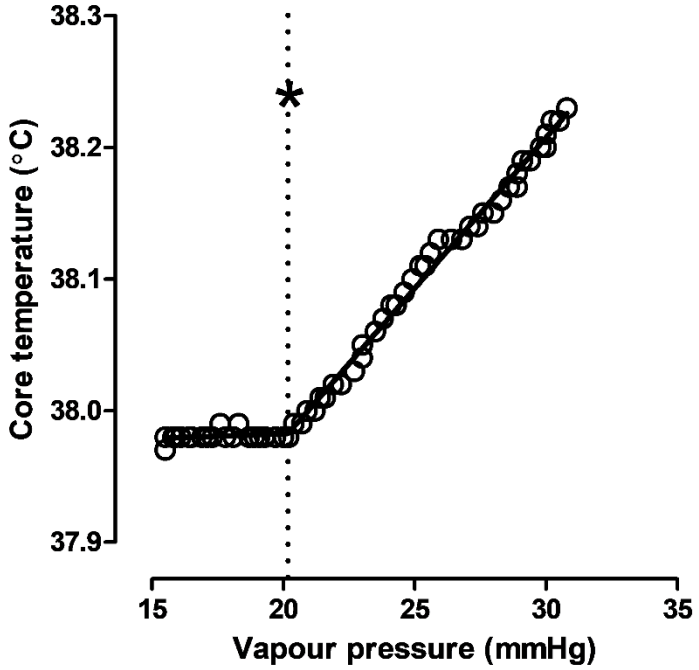


Figure 7.3. The ambient vapour pressure at the point of upward inflection in core temperature. *Point of inflection.

During exercise, $\dot{V}O_2$ was measured using the same breath-by-breath gas analyser. Heart rate was recorded throughout (Polar Heart Rate Monitor M400, Warwick, UK). Rating of perceived

exertion was recorded using a 6-20-point Borg scale (Borg, 1982), while TC was recorded using a 7-point scale (where -3 = “much too cool”, 0 = “comfortable” and 3 = “much too warm” [Bedford, 1936]). Thermal sensation was recorded using a 9-point scale (where -4 = “very cold”, 0 = “neutral” and 4 = “very hot” [Zhang et al., 2004]). Perceptual data (RPE, TC and TS) were recorded at 5 min intervals during the rest and exercise periods.

7.3.4.3. Sweating measurements

Participants’ body mass was measured at multiple timepoints during the trial. Given the nature of the exercise trials, participants’ body mass was recorded whilst wearing cycling shorts, a sports bra for females, a HR monitor, with the inserted rectal thermistor and the skin thermistors fitted to the skin. Whilst this added some mass to the participant, this was consistent throughout all trials. A force plate (Type 1758A10, Kistler Instruments Ltd, Farnborough, UK) was used at sampling frequency of 1000 Hz and had a CV of 0.05% for body mass measurements. Participants’ body mass was measured in the environmental chamber, on a hard, flat surface, immediately pre-exercise and post the 45 min fixed-humidity and 60 min ramped-humidity exercise periods.

Local sweat rate was determined using the absorbent patch technique on the left scapula, via the method reported in Chapter Six. Measurements were taken during the final 5 min of the 45 min fixed-humidity exercise period and the 60 min ramped-humidity exercise period. The patch (Medipore + Pad [3M]) was 5 cm x 5.5 cm, with an absorbent capacity of ~7 g. It was weighed (resolution 0.01 g; Ohaus, Navigator N24120, Nänikon Switzerland) prior to and after the 5 min skin application. Local sweat rate (mg/cm^2/min) was determined using equation 26.

$$\text{Local sweat rate} = \frac{\text{pre to post change in patch mass (mg)}}{[5\ (\text{cm}) \times 5.5\ (\text{cm})] \times 5\ (\text{min})}\ [\text{mg/cm}^2/\text{min}] \quad \text{(equation 26)}$$

The modified iodine-paper technique was used to determine SGA on the right scapula, using the method detailed in Chapter Six. In brief, 100% cotton paper (Southworth, Agawam, MA, US) was cut to 9 x 9 cm, saturated with iodine in the preceding 24 h, and then applied to the skin for 5 s at the end of the 45 min fixed-humidity exercise period and the 60 min ramped-humidity exercise period. As recommended in Chapter Six, to establish SGA, the optimal area of sweat gland density within 3 x 3 cm and 1 x 1 cm areas within the 9 x 9 cm iodine paper area was determined. The optimal area was defined as the area (3 x 3 cm and 1 x 1 cm) with the highest density of recruited glands.

7.3.4.4. Partitional calorimetry

As detailed in Chapter Three, heat balance parameters, such as $\dot{H}_{prod}$ (equation 1), evaporative requirement for heat balance ($\dot{E}_{req}$; equation 6), evaporation at the skin surface ($\dot{E}_{skin}$; equation 17) and heat storage (S; equation 21) were estimated via partitional calorimetry (Cramer & Jay, 2019). $\dot{H}_{prod}$ was also expressed relative to BSA (DuBois & DuBois, 1916).

$$\dot{E}_{req} = \dot{H}_{prod} - \dot{H}_{dry\ skin} - \dot{H}_{res}\ [W] \text{ (equation 6)}$$

$$\dot{E}_{skin} = \text{delta body mass loss} \times \frac{\lambda}{1000}\ [kJ] \text{ (equation 17)}$$

Where: λ is the latent heat vaporisation of sweat (2426 J/g).

$$S = \text{time} \times \frac{\dot{H}_{prod} - \dot{H}_{dry\ skin} - \dot{H}_{evap\ skin} - \dot{H}_{res}}{1000}\ [kJ] \text{ (equation 21)}$$

On the assumption that blood entering and leaving the cutaneous circulation was equal to core and T_{sk}, respectively, maximum SkBF was determined as (Sawka & Young, 2006):

$$SkBF = \frac{(\frac{1}{SH} \times \dot{H}_{prod})}{(T_{core} - T_{sk})}\ [ND] \text{ (equation 27)}$$

Where SH = specific heat of the blood (~1 kcal/°C) and $\dot{H}_{prod}$ is expressed in kcal/min.

7.3.4.5. Supplementation

After randomisation to the placebo or experimental condition, all supplements were administered in powder form within gelatine capsules, in a double-blind manner. The capsules contained either 100% isolated taurine or placebo (100% maltodextrin) and were prepared using an analytical balance (Ohaus, Navigator N24120, Nänikon Switzerland). Participants ingested the supplements for a total of eight-days, having 50 mg/kg of body mass per day of taurine or 30 mg/kg of body mass per day of maltodextrin across the eight-day period. They were requested to ingest all capsules in the morning on the days preceding the laboratory visits. On day 7 of supplementation, participants performed the incremental test and ingested the supplements 1.5 h prior to exercise. On day 8 of supplementation, the participants undertook the experimental trial and ingested the supplements 30 min before entering the environmental chamber. Supplement blinding was deemed successful, as participants only guessed which condition they were in correctly 33% of the time. The taurine dosage administered in the current study was informed by published recommendations (Waldron et

al., 2018a; Warnock et al., 2017) and because it has previously been demonstrated to be efficacious for thermal sweating during exercise in the heat (Page et al. 2019). The timing of ingestion was designed to elicit peak plasma taurine availability during exercise in both the incremental test and the experimental trial (Ghandforoush-Sattari et al., 2010). Both the taurine and maltodextrin were sourced from Myprotein (Manchester, UK).

7.3.4.6. Blood sampling

Venous blood samples were taken pre- and post-trial for the measurement of plasma taurine concentration and fingertip capillary blood samples were used to estimate plasma volume changes (Dill & Costill, 1974). Both pre- and post-measurements were conducted in a cool room (~20°C). Participants were asked to sit quietly for 10 min prior to any blood sampling, as plasma volume is affected by postural changes (Hagan et al., 1978). Blood was drawn into capillary tubes and microcuvettes (Hemocue Hb 201) for the measurement of haematocrit and haemoglobin concentration, respectively. The capillary tubes were spun in a microcentrifuge (Hawksley Neuation HCT Hematocrit Centrifuge, iFuge-HCT, Hawksley & Sons Ltd., Sussex, England) at 10,000 rev/min for 5 min and separated red cell volume was measured using a haematocrit reader (Hawksley Micro-Haematocrit Reader, Hawksley & Sons Ltd., Sussex, England). All samples were taken and measured in duplicate, with the mean value recorded for analysis. Venous blood samples were obtained via venipuncture from an antecubital vein and were drawn into three ethylenediaminetetraacetic acid (EDTA) treated vacutainer tubes (6 mL). These tubes were immediately placed on ice for 15 min before being centrifuged at 3,000 rev/min for 15 min at 4°C. The plasma was pipetted into 1.5 mL eppendorfs and stored in a -80°C freezer for subsequent analysis of taurine concentration.

7.3.5. Blood analysis

Plasma taurine concentration was measured using high performance liquid chromatography (HPLC). 100 µL of plasma sample was depleted through the addition of 400 µL of methanol and vortexed for 10 min before being centrifuged at 3000 rev/min for 5 min. The supernatant was speed vacuum concentrated to dryness at 7^{o}C and reconstituted in 100 µL of 0.4 M (pH 9) sodium bicarbonate buffer before being spiked with aspartic acid standard. The samples were analysed for taurine content using an agilent 1100 system utilising a pre-column derivatisation process and utilising OPA reagent. The samples were separated using a C18 column and ran using a gradient elution of 40 mM sodium phosphate buffer (pH 7.8) and ACN:MeOH:H_2O (45:45:10) at 40^{o}C at a flow rate of 1 mL/min. Taurine that was successfully

derivatised was detected using the fluorescence detector excitation 240 nm an emissions 450 nm with a PMT gain: 10 and a peak width of 0.5 min. Peak heights were used for quantifications.

7.3.6. Statistical analysis

A two-way repeated measures analysis of variance (RM-ANOVA) was conducted with time (rest, fixed-humidity and ramped-humidity exercise periods) and condition (taurine and placebo) as the independent variables (WBSL, LSR, SGA, T_{core}, T_{sk}, TC, TS, RPE, HR, plasma [tau] concentration, $\dot{V}O_2$, $\dot{V}CO_2$ and RER). A Greenhouse-Geisser correction was applied when the assumption of sphericity was violated. *Post-hoc* analysis was conducted with Bonferroni correction to identify significant pairwise comparisons if significant interaction effects were observed. Data were checked for normality using the Shapiro-Wilk test (Shapiro & Wilk, 1965). Two-tailed paired samples *t*-tests were used to identify significant differences between trials ($\dot{V}O_{2peak}$, time to exhaustion, P_{crit}, T_{core} at P_{crit}, delta T_{sk} during ramped-humidity, $\dot{E}_{skin}$, and heat storage during fixed-humidity and plasma volume). A Wilcoxon Signed-Rank test was performed on non-parametric data (delta T_{core} during ramped-humidity, SkBF). Within each participant the delta plasma taurine concentration and the delta WBSL between conditions was established and Pearson's correlation coefficient was performed on these delta changes to identify any relationships between plasma taurine concentration and the sweating response (WBSL). Statistical analysis was conducted in SPSS (IBM SPSS Statistics for Windows, IBM Corp, Version 24.0. Armonk, New York). Data are expressed as means ± SD throughout and a significance level of $p < 0.05$ was accepted across all tests. The magnitude of effects was calculated using Cohen's *d* and partial eta squared (η_p^2) using the following criteria of 0.2 and 0.02 (small effect); 0.5 and 0.13 (medium effect); and 0.8 and 0.26 (large effect) to denote differences, respectively (Cohen, 1988). The thresholds for the magnitudes of effects for correlations were < 0.2, 0.2, 0.5 and 0.8 for trivial, small, moderate and large effects, respectively (Cohen, 1988).

7.4. Results

7.4.1. Thermo-physiological responses

P_{crit} was greater in the taurine condition compared to placebo ($t_{(13)} = 3.817$, $p = 0.002$, Cohen's $d = 0.97$; Figure 7.4). However, there was no difference in T_{core} at the point of inflection ($t_{(13)} = -0.046$, $p = 0.964$, Cohen's $d = -0.01$) or in delta T_{core} during the ramped-humidity exercise period ($p = 0.624$) between conditions.

At rest and during the fixed-humidity exercise period, T_{core} increased with time across both conditions ($F_{(1.508, 21.117)}$ = 236.585, p < 0.001, η_p^2 = 0.944); however, there was no main effect of condition ($F_{(1, 14)}$ = 0.398, p = 0.538, η_p^2 = 0.028) or an interaction between condition and time ($F_{(1.394, 19.103)}$ = 0.394, p = 0.601, η_p^2 = 0.027). During the fixed-humidity exercise period, mean T_{sk} increased with time in both conditions ($F_{(1.150, 16.106)}$ = 15.779, p < 0.001, η_p^2 = 0.530); however, there was no main effect of condition ($F_{(1, 14)}$ = 0.975, p = 0.340, η_p^2 = 0.065) or an interaction effect with time ($F_{(1.227, 17.173)}$ = 3.668, p = 0.065, η_p^2 = 0.208). There was also no difference in the change in mean T_{sk} within the ramped-humidity exercise period ($t_{(14)}$ = -0.435, p = 0.670, Cohen's d = -0.08). Heart rate increased with time in both conditions during the whole trial ($F_{(1.281, 17.937)}$ = 95.916, p < 0.001, η_p^2 = 0.873); however, there was no main effect of condition ($F_{(1, 14)}$ = 2.134, p = 0.166, η_p^2 = 0.132) or an interaction effect ($F_{(2.354, 32.956)}$ = 3.101, p = 0.051, η_p^2 = 0.181; Figure 7.5).

There was no difference in $\dot{V}O_{2peak}$ (Taurine = 50.6 ± 8.0 mL/kg/min; Placebo = 50.1 ± 7.8 mL/kg/min; $t_{(14)}$ = 1.886, p = 0.080, Cohen's d = 0.08) or time to exhaustion (Taurine = 18.38 ± 2.1 min; Placebo = 18.33 ± 2.2 min; $t_{(14)}$ = 0.490, p = 0.632, Cohen's d = 0.01) taken during the incremental walking test in visits 2 and 4 between conditions. During the fixed- and ramped-humidity exercise periods there was no difference between conditions for $\dot{V}O_2$ (Taurine = 1324 ± 126 mL/min, Placebo = 1338 ± 126 mL/min; Taurine = 1353 ± 122 mL/min, Placebo = 1340 ± 130 mL/min, respectively; $F_{(1, 14)}$ = 0.000, p = 1.000, η_p^2 = 0.000), $\dot{V}CO_2$ (Taurine = 1166 ± 130 mL/min, Placebo = 1185 ± 125 mL/min; Taurine = 1145 ± 122 mL/min, Placebo = 1136 ± 125 mL/min, respectively; $F_{(1, 14)}$ = 0.070, p = 0.796, η_p^2 = 0.005) or RER (Taurine = 0.88 ± 0.04, Placebo = 0.89 ± 0.04; Taurine = 0.85 ± 0.03, Placebo = 0.85 ± 0.04, respectively; $F_{(1, 14)}$ = 1.764, p = 0.205, η_p^2 = 0.112). There was also no time x condition interaction effect for $\dot{V}O_2$, ($F_{(1, 14)}$ = 3.264, p = 0.092, η_p^2 = 0.189) $\dot{V}CO_2$ ($F_{(1, 14)}$ = 3.324, p = 0.090, η_p^2 = 0.192) or RER ($F_{(1, 14)}$ = 0.280, p = 0.605, η_p^2 = 0.020). However, there was a time effect for $\dot{V}CO_2$ ($F_{(1, 14)}$ = 33.736, p < 0.001, η_p^2 = 0.707) and RER ($F_{(1, 14)}$ = 48.342, p < 0.001, η_p^2 = 0.775), but not for $\dot{V}O_2$ ($F_{(1, 14)}$ = 3.395, p = 0.087, η_p^2 = 0.195).

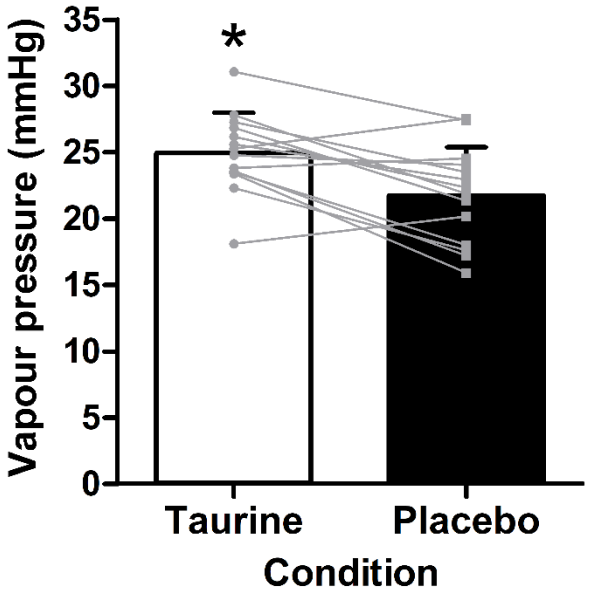


Figure 7.4. Critical ambient vapour pressure (Pcrit) - the point at which an upward inflection in core temperature was observed - indicating the transition from a compensable to an uncompensable state during the ramped-humidity exercise period in taurine and placebo conditions (mean ± SD). *Significantly greater than placebo ($p < 0.05$).

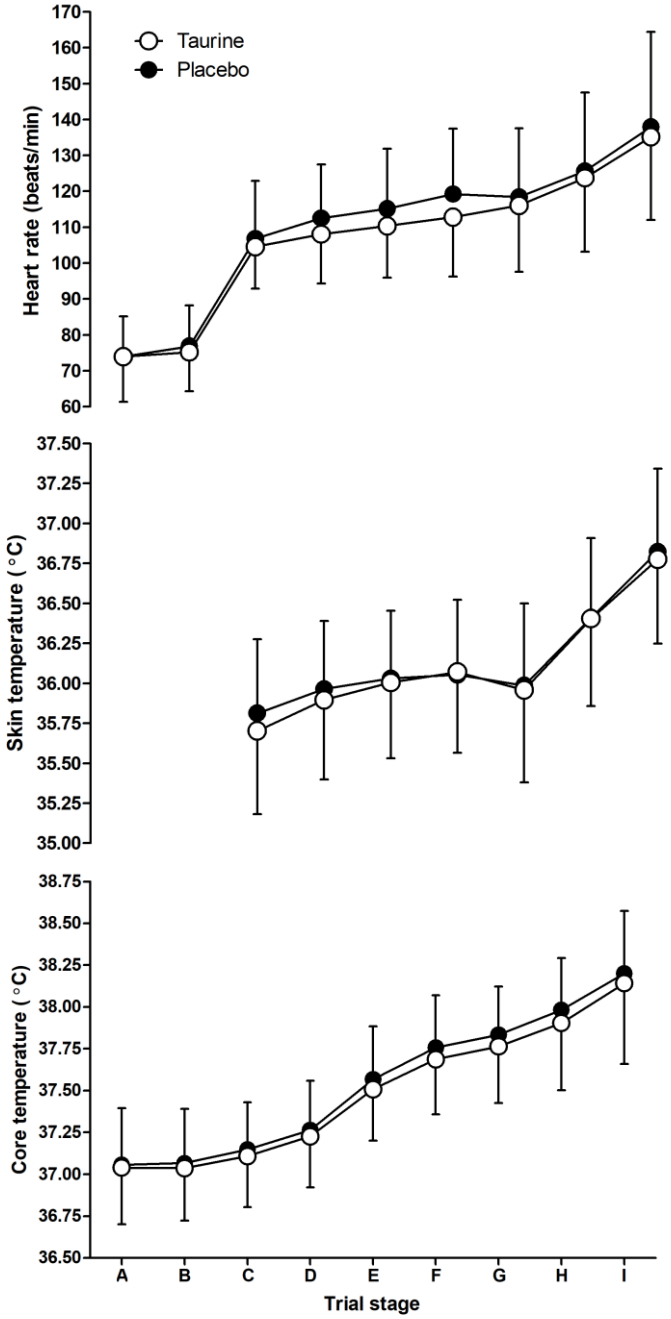


Figure 7.5. Heart rate (top), skin temperature (middle) and core temperature (bottom) plotted across time in the experimental trials (mean ± SD). **A**, Start; **B**, Rest mean; **C**, Fixed-humidity start; **D**, Fixed-humidity stage 1 mean; **E**, Fixed-humidity stage 2 mean; **F**, Fixed-humidity stage 3 mean; **G**, Ramped-humidity start; **H**, Ramped-humidity mean; **I**, End. **A** and **B** are intentionally missing for skin temperature.

7.4.2. Sweating measurements

All sweating variables are displayed in Figure 7.6. Whole-body sweat loss ($F_{(1, 14)}$ = 5.425, p = 0.035, η_p^2 = 0.279), LSR ($F_{(1, 14)}$ = 8.124, p = 0.013, η_p^2 = 0.367) and SGA (optimal 3 x 3 cm [$F_{(1, 14)}$ = 7.750, p = 0.015, η_p^2 = 0.356] and 1 x 1 cm [$F_{(1, 14)}$ = 22.525, p < 0.001, η_p^2 = 0.617]) were significantly increased in the taurine condition relative to placebo. There was a time effect for WBSL, LSR and SGA (1 x 1 cm; $F_{(1, 14)}$ = 17.438, p < 0.001, η_p^2 = 0.555; $F_{(1, 14)}$ = 35.639, p < 0.001, η_p^2 = 0.718; $F_{(1, 14)}$ = 4.806, p = 0.046, η_p^2 = 0.256, respectively), but not for SGA (3 x 3 cm; $F_{(1, 14)}$ = 4.059, p = 0.064, η_p^2 = 0.225). However, there was no condition x time interaction effect for WBSL ($F_{(1, 14)}$ = 1.607, p = 0.226, η_p^2 = 0.103), LSR ($F_{(1, 14)}$ = 0.077, p = 0.786, η_p^2 = 0.005), SGA (3 x 3 cm; $F_{(1, 14)}$ = 0.035, p = 0.854, η_p^2 = 0.003) and SGA (1 x 1 cm; $F_{(1, 14)}$ = 0.182, p = 0.676, η_p^2 = 0.013).

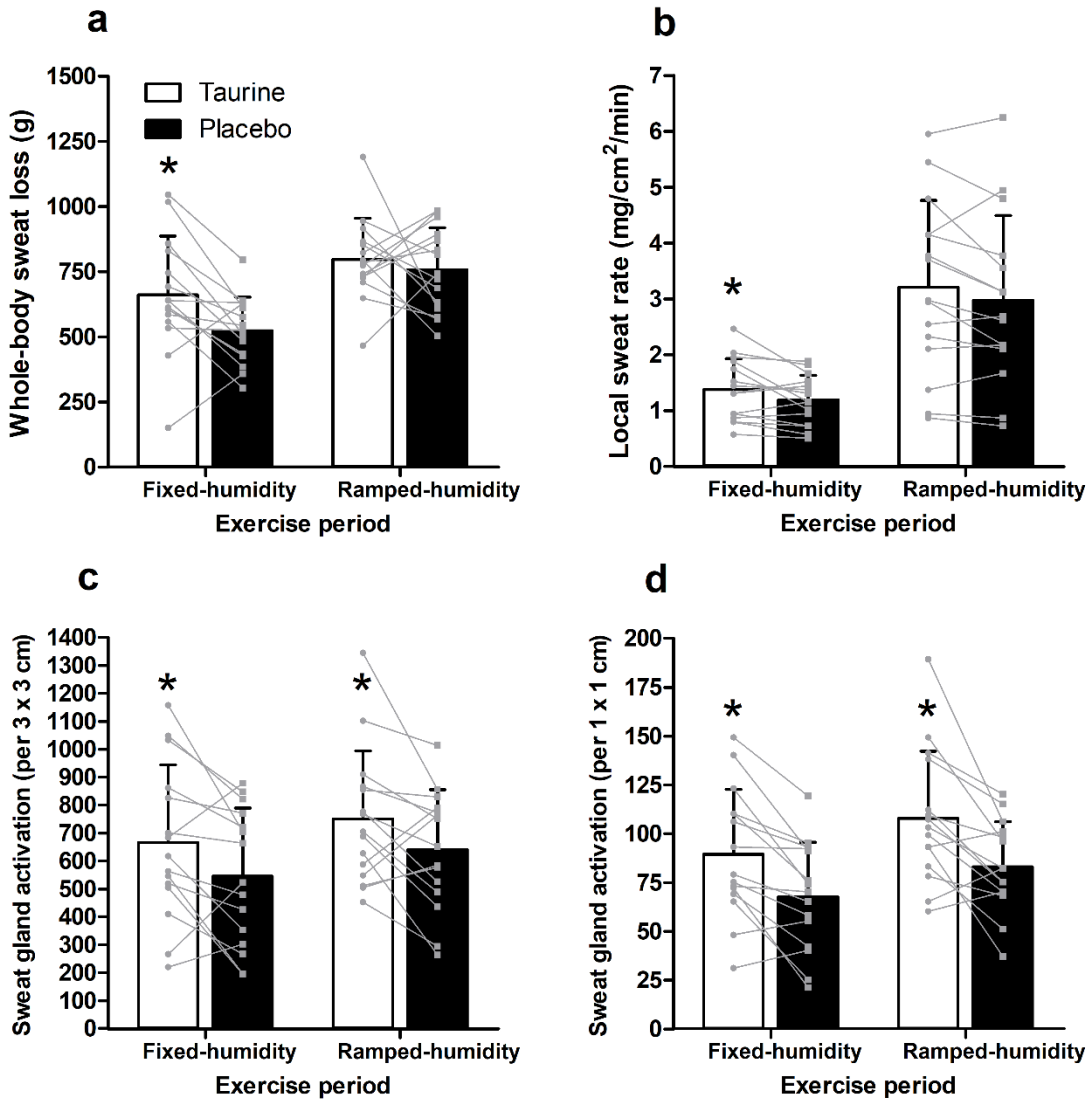


Figure 7.6. Whole-body sweat loss (a), local sweat rate (b), sweat gland activation (3 x 3 cm; c) and (1 x 1 cm; d) during fixed-humidity and ramped-humidity exercise periods (mean ± SD). *Significantly greater than placebo ($p < 0.05$).

7.4.3. Partitional calorimetry and skin blood flow

Evaporation from the skin surface ($\dot{E}_{skin}$; $t_{(14)}$ = 3.002, p = 0.010, Cohen's d = 0.79) was increased and total heat storage decreased ($t_{(14)}$ = -2.537, p = 0.024, Cohen's d = -0.87) in the taurine condition relative to placebo during the fixed-humidity exercise period (Figure 7.7). Calculated SkBF (Taurine = 4.07 ± 1.02; Placebo = 4.31 ± 1.70; p = 0.650) did not differ between conditions during the fixed-humidity exercise period.

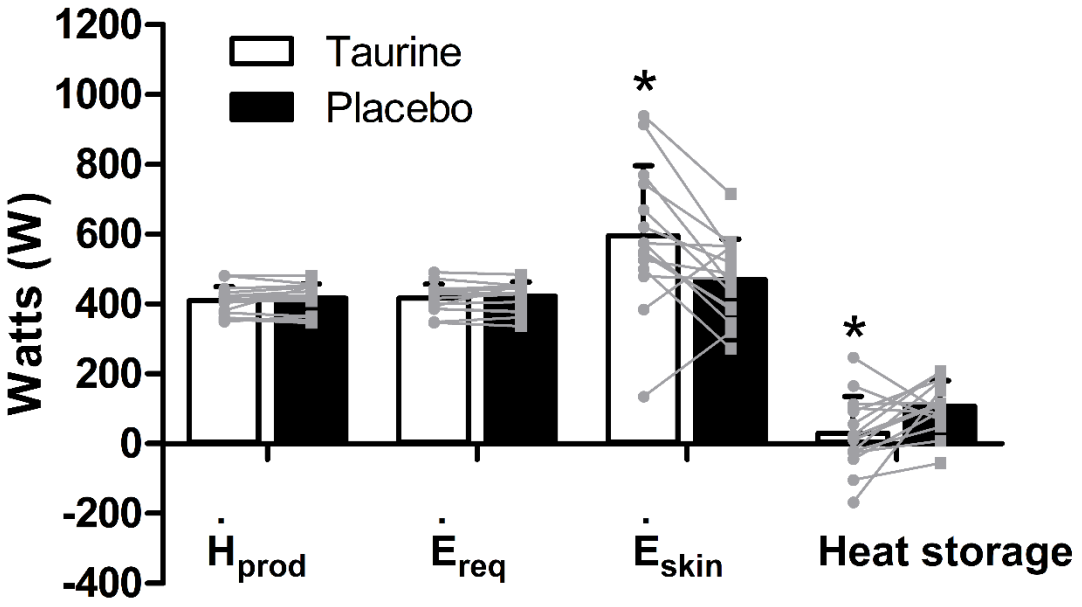


Figure 7.7. Heat production ($\dot{H}_{prod}$), evaporative requirement for heat balance ($\dot{E}_{req}$), evaporation at the skin surface ($\dot{E}_{skin}$) and heat storage during the fixed-humidity exercise period. *Significantly different than placebo ($p < 0.05$).

7.4.4. Perceptual measurements

Thermal comfort, TS and RPE increased with time in both conditions ($F_{(2.044, 28.614)} = 18.623$, $p < 0.001$, $\eta_p^2 = 0.571$; $F_{(1.901, 26.618)} = 22.955$, $p < 0.001$, $\eta_p^2 = 0.621$; $F_{(1.834, 25.669)} = 28.562$, $p < 0.001$, $\eta_p^2 = 0.671$, respectively); however, there was no main effect for condition ($F_{(1, 14)} = 0.329$, $p = 0.575$, $\eta_p^2 = 0.023$; $F_{(1, 14)} = 1.072$, $p = 0.318$, $\eta_p^2 = 0.071$; $F_{(1, 14)} = 0.639$, $p = 0.437$, $\eta_p^2 = 0.044$, respectively). There was also no interaction effect for TC ($F_{(2.681, 37.536)} = 1.753$, $p = 0.177$, $\eta_p^2 = 0.111$) or TS ($F_{(3.263, 45.686)} = 1.800$, $p = 0.156$, $\eta_p^2 = 0.114$); however, there was for RPE ($F_{(2.445, 34.225)} = 3.910$, $p = 0.023$, $\eta_p^2 = 0.218$). Post-hoc pairwise analysis revealed that RPE was only higher in the taurine condition in the last stage of the humidity-ramp exercise period (Taurine = 11 ± 2; Placebo = 10 ± 2; $p = 0.035$).

7.4.5. Plasma volume and plasma (tau) concentration

Change in plasma volume across the experimental trial (i.e. rest and fixed- and ramped-humidity exercise periods; Taurine = 0.19 ± 8.44%; Placebo = -1.86 ± 7.67%; $t_{(14)} = 0.903$, $p = 0.382$, Cohen's $d = 0.25$), did not differ between conditions. Plasma taurine concentration, both corrected and uncorrected for plasma volume, was higher ($F_{(1, 10)} = 5.266$, $p = 0.045$, $\eta_p^2 = 0.345$; $F_{(1, 10)} = 6.389$, $p = 0.030$, $\eta_p^2 = 0.390$, respectively) in the taurine *vs* placebo condition.

There was a condition x time interaction effect for corrected and uncorrected plasma taurine concentration ($F_{(1, 10)}$ = 20.212, p = 0.001, η_p^2 = 0.669; $F_{(1, 10)}$ = 20.918, p = 0.001, η_p^2 = 0.677, respectively). Post-hoc pairwise analysis revealed that both corrected and uncorrected plasma taurine concentration was only higher in the taurine condition post-trial (254 ± 198 μM *vs* 82 ± 59 μM, p = 0.011; 257 ± 183 μM *vs* 87 ± 70 μM, p = 0.006; Figure 7.8). There was no correlation between delta corrected plasma taurine concentration between conditions and delta WBSL between conditions (r = -0.43, p = 0.167).

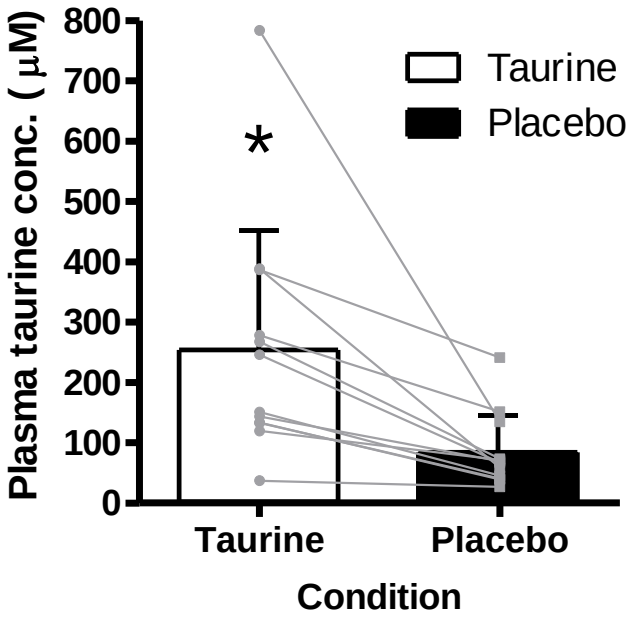


Figure 7.8. Corrected plasma taurine concentrations. *Significantly different than placebo (p < 0.05).

7.5. Discussion

The effects of eight-days oral taurine supplementation on sweating responses, T_{core}, calorimetric heat transfer components and delta plasma volume during prolonged low-intensity exercise of a fixed $\dot{H}_{prod}$ at both fixed and incremental ambient vapour pressure was investigated. In acceptance of the hypothesis, taurine supplementation increased parameters of sweating (WBSL, LSR and SGA), which delayed the upward inflection of T_{core}, denoted by a greater P_{crit} in the taurine condition compared to placebo. However, contrary to the hypothesis, there was no effect on plasma volume or T_{core} and the augmentation of the

sweating response appeared to occur earlier following exercise onset, with a trend for larger changes in WBSL and LSR observed during the fixed-humidity compared to the ramped-humidity exercise period. Despite these latter results, calorimetric modelling during fixed-humidity exercise demonstrated increased latent heat dissipation ($\dot{E}_{skin}$) and, consequently, decreased heat storage in the taurine condition compared to placebo, which is of critical importance to the findings of the current study. Thus, oral taurine supplementation appears to acutely elicit thermoregulatory benefits during low-intensity, fixed $\dot{H}_{prod}$ exercise in the heat, which is related to enhanced evaporative heat exchange, secondary to an accentuated sweating response early in the exercising period.

The increases in WBSL (~26.6%) demonstrated during the initial fixed-humidity exercise period are similar to changes reported in WBSL in response to heat acclimation (23%; Poirier et al., 2016), thus suggesting that oral taurine supplementation also stimulates a systemic sweating response. While increases in LSR (~15.5%) were considerably lower than demonstrated with heat acclimation (30%; Ravanelli et al., 2018), it is comparable with the 12.7% increase reported during higher intensity cycling in the heat following an acute taurine dose (Page et al., 2019). Interestingly, a greater recruitment of sweat glands was found in the current study following taurine supplementation compared to placebo (1 x 1 cm [32.2%] and 3 x 3 cm [22.1%]), which is in a similar range to the changes reported following eight-days of heat acclimation involving 90 min treadmill walking at 70% maximum HR in environmental conditions of 38°C and 65% RH (27.9%; Ravanelli et al., 2018). These changes observed using the absorbent patch and modified iodine-paper techniques are above their established CV% (12.8 ± 4.8% & 15.9 to 24.1 ± 9.6 to 10.8%, respectively) when exercising at 200 W/m^2 $\dot{H}_{prod}$ (Chapter Six), demonstrating a genuine increase in the response. It is noteworthy that the augmented sweating responses reported herein during low-intensity exercise occurred prior to any substantial changes in T_{core}, indicating a marked temporal mismatch between the internal thermal stimulus and the enhanced sudomotor responses. However, it should be noted that changes in T_{core} and sweating do not always correspond precisely, due to interplay with additional drivers of the sweating response (Bain et al., 2011; Cramer et al., 2012; Jay et al., 2011). Indeed, there were substantially lower effects of taurine on WBSL (5.1% increase), LSR (7.8% increase) and SGA (1 x 1 cm, 29.9%; 3 x 3 cm, 17.1%) during the ramped-humidity exercise period, compared to earlier in the trial. Therefore, advancing upon previous reports (Page et al., 2019), this study demonstrates that oral taurine supplementation stimulates early onset sweating through increased recruitment of sweat glands during exercise in the heat. This apparent early onset of sweating is somewhat different to strategies that have established effects on thermal sweating, such as heat acclimation, where greater magnitude and more sustained sweating responses are apparent (Ravanelli et al., 2018). Nevertheless, the

potential importance of this finding to human thermoregulation is underscored by the translation to an improved evaporative cooling capacity ($\dot{E}_{skin}$) in the taurine condition compared to placebo (595 W *vs* 470 W; 27% difference) and lowered rate of heat storage (30 W *vs* 108 W; 72% difference).

Owing to the nature of the current experimental protocol, it was possible to determine the effect of taurine supplementation on the breakpoint of compensability during the ramped-humidity exercise period. This threshold is important because, without changes to the exercising intensity or thermal stress, an early transition (such as that found with unacclimated people; Ravanelli et al., 2018) will lead to progressive increases in T_{core} until critical temperatures are reached. It was revealed that taurine delayed the point of uncompensable heat stress, denoted by a rightward shift in the P_{crit} value (Figure 7.4; 25.0 mmHg *vs* 21.7 mmHg). A compensable state was maintained to a higher ambient vapour pressure, most likely due to the earlier greater evaporative cooling. Therefore, the early effects of taurine on evaporative heat transfer in the fixed-humidity trial appear to cause a latent enhancement in heat tolerability, which manifested only in response to a progressive increase in the ambient humidity during the ramped segment. Despite this, T_{core} was not significantly different between conditions at the breakpoint of compensability (P_{crit}) or at any timepoint throughout the trial. There was, however, a trend for a lower T_{core} in the taurine condition during both exercise periods, indicating a sustained reduction in heat storage, as demonstrated above.

A novel aspect, and advantage, of the current study was the experimental control of $\dot{H}_{prod}$ and $\dot{E}_{req}$, which are known drivers of thermal sweating (Cramer & Jay, 2014, 2016; Gagnon et al., 2013; Chapter Six). This control of the work intensities, metabolic profile and environmental constraints was important, since it is feasible that the eight-day taurine supplementation period could have affected the metabolic response to exercise (Rutherford et al., 2010; Simmonds et al., 2022; Zhang et al., 2004) and subsequent self-paced work-rates if other exercise models were adopted. Given that the supplementation periods occurred prior to the fixed-humidity and ramped-humidity trials, poor control of these factors would have been sufficient to explain any changes in sweating response. That taurine supplementation did not meaningfully change $\dot{V}O_{2max}$ or the $\dot{V}O_2$-WR relationship in the incremental tests that preceded the heat trials, as well as maintaining its effect on thermal sweating responses under control of $\dot{H}_{prod}$ and $\dot{E}_{req}$, experimentally rules out changes in whole-body metabolism as a viable explanation. However, it remains possible that taurine elicited an endurance training-like effect, as reported previously (Waldron et al., 2018a; Waldron et al., 2019; Zhang et al., 2004) that was not recognised by the limited assessment of these characteristics herein. Indeed, endurance training is known to induce partial heat acclimation (Kobayashi et al., 1980), with lower T_{core}, increased SGA and sweat rates following 8-weeks of aerobic training (Ravanelli et al., 2018). Therefore, it remains

possible that a currently unrecognised enhancement of the endurance phenotype is partly responsible for a change in the sweating response after taurine supplementation.

Given the involvement of numerous physiological mechanisms in stimulating eccrine sweat production (Shibasaki & Crandall, 2010) and the wide-spread bioavailability of taurine (Huxtable, 1992), there are some potential mechanisms that require further investigation. A primary biological role of taurine is as an osmolyte (Cuisinier et al., 2002; Huxtable, 1992), with TauT ubiquitously expressed in many tissues, including the kidney (Baliou et al., 2020; Han et al., 2000; Han et al., 2006; Ito et al., 2010). Thus, taurine has potential to affect fluid regulation at the cellular and organ level. Indeed, exercise-induced changes in endogenous plasma taurine concentrations are related to osmoregulatory function during endurance exercise (Cuisinier et al., 2001; Ward et al., 1999), where it is actively extruded from skeletal muscle cells (Graham et al., 1991; Graham et al., 1995) to maintain intracellular osmolality (Lang et al., 1998; Sejersted & Sjøgaard, 2000; Stutzin et al., 1999). However, endogenous taurine (i.e. without oral supplementation) has not been reported to affect plasma volume (Cuisinier et al., 2002) and, herein, no difference in the plasma volume changes across the exercise period was found. However, given that the taurine condition lost a greater amount of fluid through sweating, and fluid ingestion was equal between conditions, the matching of plasma volume changes between conditions perhaps indicates a regulatory role of exogenous taurine in maintaining plasma volume, despite the additional fluid losses. Given that taurine is extruded from myocytes to the extracellular space during exercise to prevent cell swelling (Stutzin et al., 1999), a greater osmotic gradient and fluid availability in the extracellular compartments to maintain plasma volume is entirely feasible. The consequence of these findings on all fluid compartments during exercise in the heat is uncertain, however, and requires further research. It is unfortunate that plasma volume measurements were not more frequent, as earlier transient changes might have occurred in tandem with the early sweating onset but will not have been identifiable. Theoretically, plasma volume maintenance may have augmented sweating via preservation of SkBF (Nagashima et al., 1998; Nielsen et al., 1984), fluid availability and supply to the sweat gland (Fortney et al., 1981; Wong & Hollowed, 2017) or a change in osmoreceptor or baroreceptor signalling (Mack et al., 1995; Shibasaki, Kondo, et al., 2003).

Estimated whole-body SkBF was not different between conditions. This result was unanticipated, as taurine acts peripherally, as a vaso-relaxant, with TauT abundantly expressed in vascular smooth muscle (Liao et al., 2007). Supplementation has been demonstrated to improve both endothelium-dependant and independent vasodilation (Maia et al., 2014; Sun et al., 2016; Ulusoy et al., 2017), through increased NO bioavailability, restoration of vascular redox homeostasis and calcium activated potassium channel opening

action (Maia et al., 2014; Ulusoy et al., 2017). Its apparent homeostatic function appears to play a role in vascular tone, by promoting both vasodilation and vasoconstriction, to increase blood flow during ischemia, hypoxia or heat stress and maintain blood pressure (Nishida & Satoh, 2009). An association between sweating and SkBF has been established (Brengelmann et al., 1973; Nadel et al., 1971; Nadel et al., 1971; Van Beaumont & Bullard, 1965), suggesting a functional inter-relationship (Wong & Hollowed, 2017). However, findings by Ravanelli et al. (2017) demonstrate that increases in SkBF are not a prerequisite for increases in LSR, at least acutely. This is supported by the results herein, as the similarity in estimated whole-body SkBF between the taurine and placebo condition demonstrates no apparent vascular effect, despite the concomitant increased sweating response. While this may be the case, attenuation of SkBF through arterial occlusion (Collins et al., 1959; MacIntyre et al., 1968) or pharmacological blockade (Wingo et al., 2010) reduces the sweating response during heat stress, suggesting a requirement of SkBF for sustained sweating. Future analysis of cutaneous vascular conductance (CVC) using laser doppler flowmetry would more conclusively establish whether additional SkBF is required to continue to supply the sweat gland during prolonged periods of sweating and whether taurine supplementation facilitates this.

This study characterised multiple measures of the sweating response (WBSL, LSR and SGA), which demonstrated changes of a large magnitude after taurine supplementation. This enhancement occurring in the earlier segments of the current trial, prior to any marked heat strain, requires mechanistic reasoning and may be explained by a centrally-mediated alteration in thermoregulatory set-point. Indeed, whilst taurine supplementation did not significantly affect T_{core}, there were clear effects on the domain of compensability in the current study, and the enhanced early sweating response occurred alongside increased sweat gland recruitment, providing further evidence for an alteration in the thermoregulatory feedback loop. This has been suggested previously in exercising humans to explain early onset of sweating in response to high-dose (50 mg/kg) taurine supplementation (Page et al., 2019). Additionally, another potential mechanism for the increased sweating observed is through taurine antagonism of ADH or AVP. Vasopressin is an antidiuretic hormone produced in the supraoptic nucleus of the hypothalamus and released by the posterior pituitary gland in response to plasma hyperosmolality (Bourque et al., 1994; Cunningham & Sawchenko, 1991; Richard & Bourque, 1995). Both potential mechanisms can be linked to taurine's role as a neuromodulator, where it acts as a glycine and GABA receptor agonist and appears to have multiple roles in maintaining homeostasis during periods of perturbation (Hussy et al., 1997; Jia et al., 2008; Schmieden et al., 1992). Indeed, it functions to protect neurons from toxicity by modulating thalamic network activity under conditions of homeostatic derangement, which

are associated with severe pathological conditions (Jia et al., 2008) and may be extended to transient states of heat stress. It has been identified in the animal model that GABA and taurine are released from some hypothalamic cells into the cerebrospinal fluid during thermal strain, which coincides with reductions in T_{core} (Frosini et al., 2000). Following oral supplementation, the increased plasma taurine is available to cross the blood-brain barrier via TauT (Kang, 2002), and act on hypothalamic regions of the brain, potentially interacting with a specific taurine binding site (i.e. putative Taurinergic pathway) or GABA receptors (Frosini et al., 2003; Quéva et al., 2003). Whilst it remains speculative, in the exercising, thermally-stressed human, it is proposed that the established cryogenic effect of these pathways (Elhussiny et al., 2021; Frosini et al., 2003) may translate to enhanced sudomotor function, as the major effector response to heat stress. Similarly, taurine's release from the hypothalamus in response to plasma hypoosmolality and its agonism of glycine receptors is suggested to exert an inhibitory effect on ADH secretion (Deleuze et al., 1998; Hussy et al., 1997; Miyata et al., 1997), thereby promoting increased fluid loss. Due to their many similarities, it has been suggested that ADH may facilitate fluid reabsorption at the sweat gland, as it does in the kidney, through its action on V2 receptors, promoting fluid and sodium reabsorption to maintain fluid homeostasis (Agu, 2017; Baker, 2019; Hew-Butler, 2010). As such, exogenous taurine supplementation may supress the release of ADH and attenuate water reabsorption at the sweat gland, leading to greater fluid loss. In the rat model subcutaneous injection of ADH reduced initial sweat rate by 50%, suggesting a role for ADH in regulating the sweating response (Quatrale & Speir, 1970). Further, in exercising humans a positive association between plasma ADH and sweat sodium concentrations has been reported, indicating it may have a potential role in fluid retention at the sweat gland (Hew-Butler, 2010). Nevertheless, several studies both augmenting and suppressing ADH have observed no significant change in sweat rate during exercise or heat exposure (Allen & Roddie, 1974; Gibiński et al., 1979; Hew-Butler et al., 2014; Pearcy et al., 1956; Ratner & Dobson, 1964; Senay & Van Beaumont, 1969; Taussig & Braunstein, 1973). However, none of these studies have investigated the effect of ADH on the sweating response during exercise in the heat when fluid intake is regulated, and other drivers of the sweating response are controlled (e.g. $\dot{H}_{prod}$ and $\dot{E}_{req}$). Therefore, this remains a plausible pathway in which exogenous taurine supplementation augments the thermal sweating response and requires further investigation. Whilst the above mechanisms of action provide a potential explanation for the current results, it is reasonable to suggest that the increased sweating response occurs owing to a combination of several factors, which is consistent with the numerous biological roles ascribed to taurine (Huxtable, 1992). *In-vivo* investigation of the above central mechanisms is likely to be challenging but could be addressed in future research.

High environmental heat stress alongside physical exertion where compensatory limits for heat dissipation are exceeded, pose a risk for serious heat illness due to inexorable increases in T_{core} (Epstein & Yanovich, 2019; Howe & Boden, 2007). Athletes and military personnel are often required to perform endurance exercise in such conditions (Ely et al., 2008; Parsons et al., 2019; Racinais et al., 2015; World & Booth, 2008) and, therefore, a strategy (e.g. taurine supplementation) to help offset this rise in T_{core}, and the risk of heat illness could be important in reducing its prevalence. To the authors' knowledge, there are no known serious side effects of taurine supplementation at doses of up to 10 g (Shao & Hathcock, 2008). Therefore, supplementing 50 mg/kg of taurine prior to heat exposure might be a useful strategy to offset the deleterious effects of heat stress among healthy populations. However, further research is required to better understand the efficacy of its thermoregulatory role in applied settings. Thus, findings that oral taurine supplementation can acutely increase the sweating response, augment evaporative heat transfer, and reduce heat storage during low-intensity exercise in the heat has many promising future applications.

7.5.1. Conclusion

Eight-days of oral taurine supplementation (50 mg/kg) increased sweating responses (WBSL, LSR and SGA) during low-intensity exercise of a fixed $\dot{H}_{prod}$ in the heat. The subsequent enhanced evaporative cooling ($\dot{E}_{skin}$) and reduced heat storage delayed the subsequent upward inflection in T_{core} - represented by a greater P_{crit} - demonstrated during the exercise period of incremental ambient vapour pressure. Despite this, there was only a trend towards a lower T_{core} throughout the trial, and reduced changes in WBSL and LSR during the ramped-humidity exercise period. This apparent early augmentation of the sweating response appears to offer thermoregulatory benefits for latter parts of an exercising period. These findings have potential implications for both athletes and military personnel performing exercise in hot environmental conditions that permit sufficient latent heat transfer. The experimental control of the thermal drivers of sweating ($\dot{H}_{prod}$ and $\dot{E}_{req}$) suggests that other mechanisms are likely to be responsible for the observed increase in sweating. This study suggests several possibilities to direct future investigations, which will help to elucidate the mechanistic actions of taurine during exercising-heat stress.

8. Chapter Eight – The effect of oral taurine supplementation on thermoregulatory and cardiometabolic responses to passive heat exposure

8.1. Abstract

Background In hot or humid environments, there is a reduced capacity for dry or evaporative cooling, which can result in uncompensable heat stress and rises in T_{core}. Cardiovascular adjustments (e.g. elevated HR, $\dot{Q}$ and peripheral vasodilation) attempt to meet heat loss demands by facilitating greater SkBF and occur in parallel to increases in sudomotor function. An individual's ability to thermoregulate (i.e. enhanced vasodilation and eccrine sweating) and, consequently, tolerate such conditions, can be chronically or acutely modified. Taurine supplementation enhances sweating onset and rate during exercise in the heat and induces greater peripheral arterial vasodilation, but its potential role in thermoregulation at rest has not been evaluated.

Objectives To determine the effect of oral taurine supplementation on sweating, vascular, cardiometabolic and T_{core} responses during 90 min passive heat exposure.

Methods Thirteen, healthy, non-heat acclimated female (*n* = 2) and male (*n* = 11) participants (26 ± 5 years, 79.3 ± 9.6 kg) completed a 90 min passive heating protocol (38.4 ± 0.4°C, RH 55.9 ± 1.9%), following eight-days of oral taurine supplementation (50 mg/kg of body mass) or placebo in a double-blind, randomised, cross-over design. Whole-body sweat loss, LSR, SkBF, cutaneous vascular conductance (CVC), brachial artery diameter and blood flow, mean arterial pressure (MAP), parameters of cardiovascular function, pulmonary gas exchange and T_{core} were assessed. Relative % SkBF and brachial artery vasodilation (V%) were analysed during and post isometric handgrip exercise, respectively, at three timepoints (20, 50 and 80 min). Plasma taurine concentrations were determined from post-supplementation blood samples.

Results Taurine supplementation increased WBSL by 16.2% (p = 0.049), leg LSR by 26.6% (p = 0.011), SkBF by 19.3% (p = 0.016) and CVC by 9.3% (p = 0.027) compared to placebo. Post-isometric handgrip brachial artery (8.0 *vs* 5.8%; p = 0.002) and SkBF (6.3 *vs* 4.6%; p = 0.020) V% were greater in the taurine condition; however, no differences in resting brachial artery diameter and blood flow, MAP, cardiometabolic parameters or T_{core} were observed (p > 0.05). Plasma taurine bioavailability was increased in taurine *vs* placebo (258 ± 55 *vs* 74 ± 26 μM).

Conclusion Taurine supplementation influenced aspects of thermoregulation during passive heat exposure, with enhanced sweating and cutaneous vasodilatory responses, without affecting other parameters of cardiometabolic function. These findings have potential implications for individuals at risk of heat stress in environmental conditions that permit dry and evaporative heat dissipation.

8.2. Introduction

Due to human influence on climate change, average surface temperatures are rising rapidly (Lee et al., 2023), with a greater occurrence, severity and duration of heatwaves across the globe. This is exposing an increasingly larger proportion of the population to extreme heat (Marx et al., 2021). Indeed, even seemingly small (1 to 2°C) increases in average summer WBGTs can be sufficient to induce heat strain (Pal & Eltahir, 2016; Sherwood & Huber, 2010), which can lead to adverse health outcomes (Zhang et al., 2020). Prolongation of these heat exposures puts individuals at increased risk of heat syncope, heat exhaustion, heat stroke, cardiovascular events and even death (Liu et al., 2022; Székely et al., 2015).

The vast majority of heat-related mortalities are of cardiovascular origin (Gasparrini & Armstrong, 2011; Jingesi et al., 2023; Liu et al., 2022; Pradhan et al., 2019; Weisskopf et al., 2002). Whilst physical activity in the heat acutely exacerbates the probability of hyperthermia and heat-related illnesses (Coris et al., 2004; Lundgren et al., 2013; Nadel et al., 1977), adverse health events occurring as a result of thermal strain have also been reported during periods of inactivity or rest in hot and/or humid conditions (Ballester et al., 2023). Compensable heat stress occurs when metabolic heat can be sufficiently dissipated into the environment, leading to balanced heat storage and maintenance of core temperature (Cramer & Jay, 2016). However, in hot environmental temperatures and/or high RH, there is a reduced capacity for dry (conductive, convective and radiative) and evaporative cooling ($\dot{E}_{skin}$), respectively (Che Muhamed et al., 2016), which often results in uncompensable heat stress and rises in T_{core}. Thus, in thermally stressful environments, strain is placed upon the cardiovascular system to facilitate sufficient $\dot{Q}$ to adequately perfuse all metabolically active tissues, including the skin, to support dry heat loss and evaporative cooling (González-Alonso, 2012; Rowell, 1974). In young, healthy individuals, these demands can often be met, assuming ambient conditions are not too extreme (i.e. uncompensable conditions), via cardiovascular adjustments (elevated HR and $\dot{Q}$ and peripheral vasodilation), facilitating greater SkBF (Crandall & Gonzalez-Alonso, 2010; Crandall & Wilson, 2015), which occur in parallel with increases in sudomotor function (Gagnon & Crandall, 2018). However, there is substantial inter-individual variability in thermoregulatory capacity, even among healthy people

(Cramer & Jay, 2015; Lind et al., 1970), with many demonstrating inferior thermoregulatory capacity and, thus, greater risk of heat related illness. Therefore, interventional strategies capable of potentially offsetting heat strain in response to periods of thermal stress are of current relevance.

An individual's ability to thermoregulate (i.e. enhanced vasodilation and eccrine sweating), and, consequently, tolerate hot environmental conditions, can be chronically or acutely modified through various interventions (Périard et al., 2021). Improved SkBF, SGA, as well as the rate and onset of sweating, have been demonstrated in response to exercise interventions or heat acclimation, comprising 8 to14 days of heat exposures (Klous, De Ruiter, Alkemade, Daanen, et al., 2020; Lorenzo et al., 2010; Poirier et al., 2016; Ravanelli et al., 2018), with hallmark adaptations reported to decay at ~2.5 %/day after removal of the thermal stimulus (Daanen et al., 2018). Whilst these approaches demonstrate the plasticity of thermoregulatory capacity, there are several, more acute, interventions, such as the use of dietary supplements, which might confer benefits when implemented in hot conditions (Chapter Four). Of the supplements previously reviewed, oral taurine was reported to elicit substantial increases in sweating and corresponding reductions in T_{core} responses compared to placebo conditions (Page et al., 2019; Chapter Seven), thereby lowering heat storage.

Taurine is a sulphur containing amino acid, found in most animal products such as eggs, meats and seafood (Abebe & Mozaffari, 2011; Laidlaw et al., 1990). The majority of mammalian taurine is obtained from these dietary sources, but it can also be endogenously synthesised from the semi-essential amino acid cysteine in a three-step process (Bin et al., 2017). There are many physiological roles ascribed to taurine, which could have a beneficial thermoregulatory effect during heat exposure (Huxtable, 1992). For example, it appears to induce peripheral vaso-relaxation (Ulusoy et al., 2017; Yildiz & Ulusoy, 2022), with one clinical study reporting improvements in both endothelium-dependant (3.2% increase in FMD) and independent vasodilation (4.4% increase in nitro-glycerin mediated dilation) among pre-hypertensive patients (Sun et al., 2016). The potential mechanisms by which this occurs are likely to be related to improvements in NO bioavailability (endothelium-dependant vasodilation; Palmer et al., 1987) and/or the taurine-induced increase in synthesis of the signalling molecule H_2S and its precursors CSE and CBS (endothelium-independent vasodilation; Liang et al., 2011; Sun et al., 2016). Hydrogen sulphide regulates vascular tone (Siebert et al., 2008; Yang et al., 2008), through ATP-activated potassium channels (Maia et al., 2014; Ulusoy et al., 2017). Further, endogenous taurine can directly control vascular tone through modulation of Ca^{2+} and K^{+} channel function (Franconi et al., 1982), where it appears to have a homeostatic function to maintain blood flow and blood pressure. *In-vitro* studies of rodent vessels have reported that, at high intracellular Ca^{2+} concentrations associated with

hypoxic conditions, taurine inhibited Ca^{2+} channel function to induce vasodilation, with the opposite effect reported following low intracellular Ca^{2+} concentrations (Yildiz & Ulusoy, 2022). These findings might also explain the vasoconstrictive effect of taurine on the subcutaneous vasculature, *in-vivo*, during exercise and cold exposure (Simmonds et al., 2022). Therefore, it is feasible that, under conditions of resting heat stress, taurine may induce peripheral vasodilation in response to the heat-induced increase in peripheral blood flow, thereby increasing dry heat transfer capacity and fluid availability to activated sweat glands, while concomitantly lowering blood pressure.

While taurine's vasoactive role has not yet been established in the heat, its effect on sudomotor function has been somewhat characterised. Enhanced sweating onset and rate (~12.7%; Page et al., 2019), WBSL (~26.6%), LSR (~15.5%) and SGA (~22.1 to 32.2%; Chapter Seven) have been reported in response to oral taurine supplementation (50 mg/kg). Page et al. (2019) also demonstrated significant reductions in T_{core} (end T_{core} of 38.1°C *vs* 38.5°C), further suggesting a thermoregulatory role for taurine. Despite Chapter Seven reporting no reduction in T_{core}, the upward inflection in T_{core} - denoting the transition from a compensable to an uncompensable state - was delayed in the taurine condition, indicating greater heat tolerability. The mechanistic explanation for this augmented sweating response is yet to be elucidated. Nevertheless, the earlier onset of sweating is potentially indicative of a central mechanism, which could be related to its role as a neuromodulator (Hussy et al., 2000; Jia et al., 2008). Furthermore, taurine's established accumulation in the interstitial fluid (Pasantes-Morales et al., 1998) could be enhanced, secondary to oral supplementation, increasing the osmotic gradient and, thus, fluid availability for eccrine gland sweat production.

Taurine also has a central role in cardiac function (Schaffer et al., 2010), primarily regulating sarcoplasmic reticulum Ca^{+} handling and Ca^{+} sensitivity in animal cardiac muscle cells. Here, taurine improves contractile function (Schaffer et al., 2010) and has a positive inotropic effect (Satoh & Sperelakis, 1998). Further, *in-vivo*, increases in SV have been observed when taurine is ingested within an energy drink, but these data are limited owing to the inclusion of other ingredients, such as caffeine (Baum & Weiss, 2001). However, no changes in HR have been observed at rest or during exercise in the heat after oral taurine supplementation (Page et al., 2019; Chapter Seven), yet meta-analytical data demonstrates the potential for oral taurine supplementation to reduce blood pressure across healthy and clinical populations (Waldron et al., 2018b). Therefore, any central cardiovascular change elicited with taurine supplementation most likely occurs alongside peripheral vascular adjustments, but these effects have not been investigated during passive heat stress in humans. Potential increases in SV, and presumably $\dot{Q}$, are consistent with the heat acclimated phenotype (Périard et al., 2016), which most likely reflect an enhanced cardiac stability or efficiency (Horowitz, 2002).

Based on the available data, it is feasible that taurine offers a similar effect in promoting cardiac stability or efficiency during periods of cardiovascular perturbation (i.e. heat stress) but this requires more detailed characterisation.

There is promising evidence that taurine supplementation elicits a number of physiological effects that enhance thermoregulatory capacity during heat exposure; however, it is important to further evaluate this in resting conditions, in the absence of other influencing factors, such as whole-body exercise, which cause additional $\dot{H}_{prod}$ (Brotherhood, 2008), cardiovascular adjustments (Rowell, 1974; Vatner & Pagani, 1976) and influences on the sweating responses (Shibasaki & Crandall, 2010). In hot and/or humid conditions, thermoregulatory strain, and the risk of heat illness, is apparent in the resting state (Deshayes & Périard, 2023), which could be attenuated by taurine supplementation. Therefore, it is important to characterise the effect of taurine on the cardiovascular system, sweating and thermoregulation, which is currently unknown.

The aim of the current study was to determine the effect of an eight-day taurine supplementation period on T_{core}, vascular (blood pressure, SkBF, brachial artery diameter and blood flow), sweating (WBSL and LSR), cardiovascular ($\dot{Q}$, SV and HR) and metabolic (oxygen consumption at the muscle) responses and plasma taurine concentrations during passive heating. It was hypothesised that taurine supplementation would: i) induce greater sweating responses (WBSL and LSR); ii) induce peripheral vasodilation (brachial artery diameter and SkBF); iii) increase $\dot{Q}$ and SV, and lower blood pressure and; iv) lower T_{core}.

8.3. Methods

8.3.1. Participants

Thirteen non-heat acclimated, healthy females (*n* = 2) and males (*n* = 11) volunteered to take part in the study (26 ± 5 years, 178 ± 5 cm, 79.3 ± 9.6 kg). Based on the effect sizes (Cohen's *d* = 0.75) calculated using taurine to improve vascular function (Sun et al., 2016), G∗Power (Version 3.0.10; Universität Düsseldorf, Germany) was used to calculate an appropriate *a-priori* sample size of 13. Participants were asked to refrain from alcohol and caffeine consumption for 24 h and to avoid strenuous exercise and follow a consistent diet for 48 h prior to testing. They were provided with a food diary to record their food intake and asked to replicate this prior to all subsequent visits. Use of any dietary supplements, such as caffeine, was prohibited for the duration of the study. Written informed consent was obtained from all participants. Both female participants were on hormonal oral contraceptive pills. Institutional ethics approval (JP_31-01-23b; Appendix A) was provided and the study was conducted in

accordance with the 2013 Declaration of Helsinki, except for pre-registration on a publicly accessible database.

8.3.2. Design

This study adopted a double-blind, randomised, placebo-controlled, cross-over design. Participants reported to the laboratory on five separate occasions: once for pre-screening and familiarisation (visit 1), twice for blood sampling (visits 2 & 4) and twice to complete the experimental trials (visits 3 & 5). During the experimental trials, they completed a passive heating protocol, following eight-days of supplementation with either taurine (50 mg/kg) or placebo (30 mg/kg of maltodextrin; visits 3 & 5; Figure 8.1). All testing sessions took place in an environmental chamber set to a dry-bulb temperature of 38.4 ± 0.4°C and 55.9 ± 1.9% RH. The break period of 7-days between conditions was selected to permit complete recovery from the protocols and time to consume the cross-over supplementation. Taurine has a ratio of clearance/bioavailability of ~21 h and, therefore, this was considered a sufficient washout period (Ghandforoush-Sattari et al., 2010). All trials were conducted at approximately the same time of day to control for circadian rhythm. Randomisation was performed using online randomisation software (Urbaniak & Plous, 2013) by an independent person.

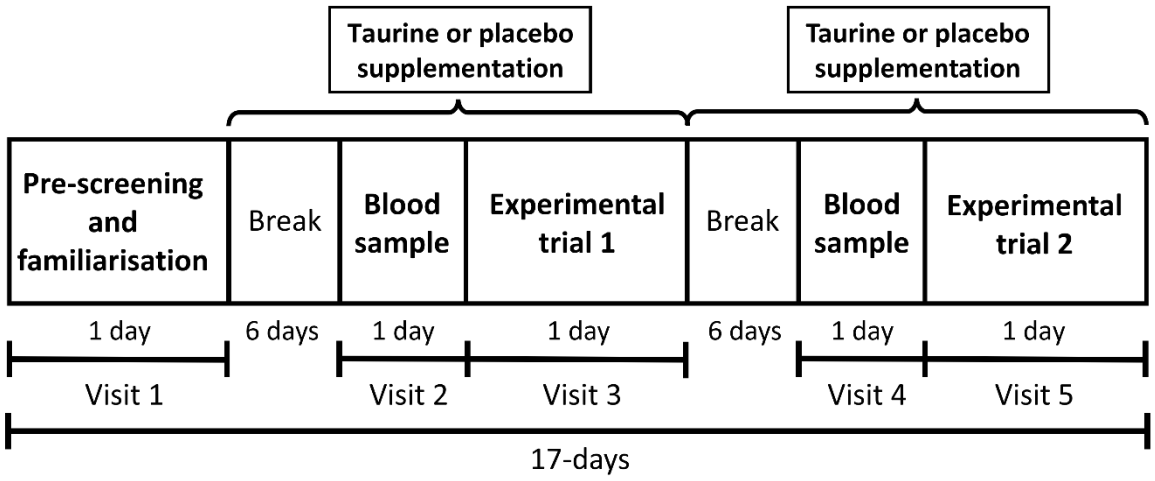


Figure 8.1. Schematic of the study timeline.

8.3.3. Experimental trials

8.3.3.1. Pre-trial instrumentation

Participants were required to arrive euhydrated, as determined by a urine osmolality value < 600 mOsm kg/H_2O (Portable osmometer, Osmocheck, Vitech, Scientific Ltd). If > 600 mOsm kg/H_2O, the participant was asked to drink 500 mL of plain water and wait 30 min. Urine osmolality was then re-determined and if the participant was deemed euhydrated, testing commenced. Participants wore running shorts (90% polyester, 10% elastane), as well as a sports bra for females. To measure T_{core}, participants were instructed to insert a flexible rectal thermistor 10 cm past the anal sphincter (Walters Medical, W0001B, England). On visit 3, participants were asked to perform two brief maximum voluntary contractions (MVCs) with their right hand using a handgrip dynamometer. The maximum value obtained was used to calculate the workload (50% MVC) to be performed by each participant at three time-points within the experimental trial.

Participants entered the environmental chamber, which was regulated to an ambient dry-bulb temperature (T_{db}) of 30.3 ± 0.2°C, RH of 51.3 ± 2.9% and lay in a supine position on a massage bed. Their right arm was positioned at 90° of shoulder abduction for instrumentation of equipment and for performing the IHG exercises. A 3-lead ECG (electrocardiogram) was placed on the participant (Jaeger Vyntus CPX, Hoechberg, Germany), with electrodes (2239 Red Dot Monitoring Electrode, 3M, St Paul MN, US) under the right and left clavicles near the shoulders and another on the lower left abdomen for the measurement of HR and the estimation of $\dot{Q}$ and SV via cardio-impedance (Drew & Funk, 2006). A continuous-wave near-infrared spectroscopy (NIRS) device (PortaMan, Artinis medical systems, Amsterdam, Netherlands) was placed on the brachioradialis muscle of the right forearm to measure muscle oxygenation during the handgrip exercise. Marker pen was used to mark its location to ensure consistent placement in the subsequent trial. The NIRS device was secured to the arm using elasticated tape and covered with a black cloth to prevent external light from disrupting the signal. Skin thermistors (Grant Instruments Ltd., Cambridge, UK) were attached to four sites on the participant's right side: upper-chest, mid-humerus, mid-calf and mid-thigh to measure weighted mean T_{sk}. Both T_{core} and T_{sk} were recorded using a data logger, continuously sampling every 5 s (SQ2010; Grant Instruments Ltd., Cambridge, UK). Ramanathan's equation (Ramanathan, 1964) was used to calculate mean T_{sk}:

$$T_{sk} = (T_{chest} + T_{arm}) \times 0.3 + (T_{thigh} + T_{calf}) \times 0.2 \ [°C] \quad \text{(equation 25)}$$

A ventilated sweat capsule system (Q-Sweat; WR Medical Electronics Co., Stillwater, MN) was used to measure LSR, with single capsules fixed proximal to the skin thermistors on the participant's right forearm, chest and thigh using flexible straps. Laser-Doppler flowmetry (Moorlab, Moor Instruments, Devon, UK) was used to estimate SkBF of the right mid-anterior forearm, with the probe affixed to the skin's surface with an adhesive ring. It was placed adjacent to the skin thermistor and ventilated capsule on an area of the forearm with no superficial veins that demonstrated pulsatile activity. Skin blood flow perfusion units were normalised to the maximum value observed for each participant during both trials. A blood pressure cuff was placed on the participant's left arm for the measurement of arterial blood pressure of the brachial artery using electrosphygmomanometry. Mean arterial pressure was calculated as diastolic blood pressure + 1/3 x pulse pressure (difference between systolic and diastolic pressure; equation 29). Cutaneous vascular conductance was calculated as perfusion units divided by MAP and expressed as a percentage of maximum (equation 28). Prior to application of all equipment, the location sites were dry-shaved. Pulmonary $\dot{V}O_2$ was measured using breath-by-breath expired gas analysis and oxygen saturation (SPO_2) was measured using a finger pulse oximeter at the left forefinger (Jaeger Vyntus CPX, Hoechberg, Germany). Environmental conditions, such as ambient T_{db} and RH were continuously monitored approximately 20 cm from the participant (Kestrel 5400 Heat Stress Tracker, Kestrel Meters, Boothwyn, PA, US; Figure 8.2).

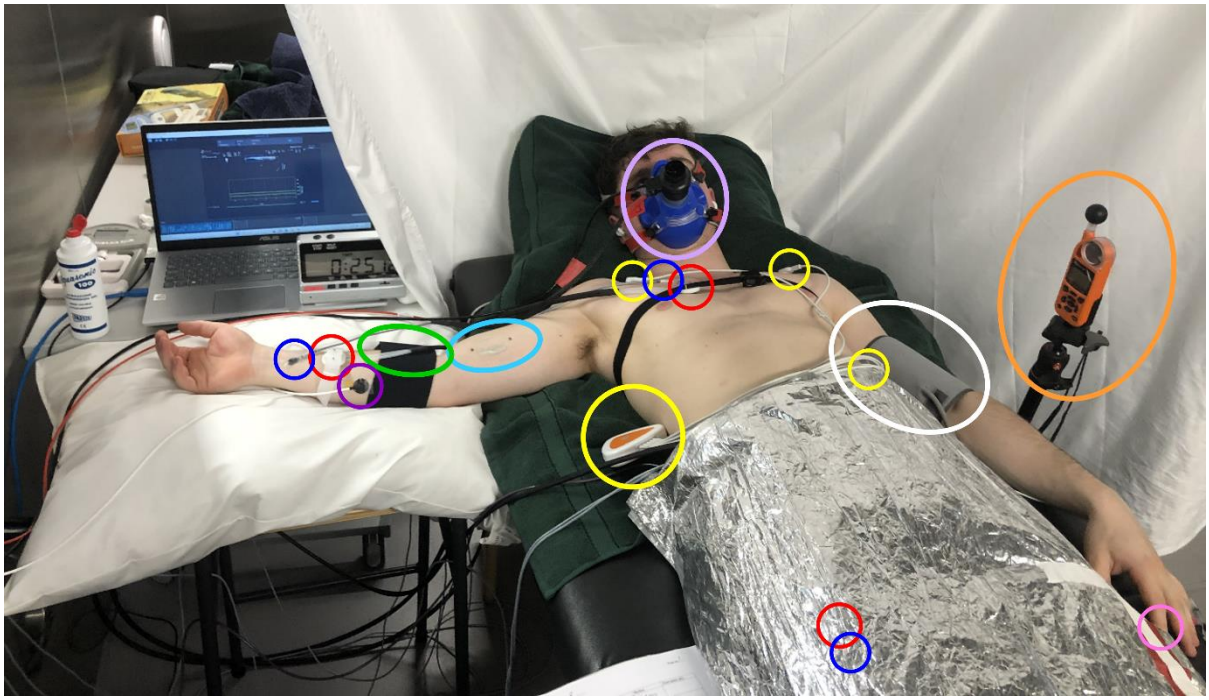


Figure 8.2. Example of the trial set-up. Cardio-impedance unit and electrodes (circled yellow), near-infrared spectroscopy unit (circled green), skin thermistors (circled dark blue), sweat

capsules (circled red), laser-doppler (circled dark purple), blood pressure cuff (circled white), expired gas analysis (circled lilac) finger pulse oximeter (circled pink) Kestrel heat stress tracker (circled orange) and brachial artery ultrasound measurement site (circled light blue).

8.3.3.2. Trial

After instrumentation, the participant rested for 10 min while baseline measurements were taken (Figure 8.3). B-mode echo and pulse-wave Doppler ultrasound (B-mode ultrasonography; MyLab9, Esaote, Genoa, Italy) with a 10 MHz probe (L 3-11 Linear Probe, Esaote, Genoa, Italy) was used to analyse and record right brachial artery diameter and blood flow velocity for 1 min (Cardiovascular suite, version 4.3.0). The probe was positioned longitudinally on the brachial artery, at the distal third of the upper arm and held in a constant position during measurement. Upon trial commencement, the temperature of the chamber was increased to 40°C and the participants legs were enclosed in a reflective foil blanket to aid in inducing passive heat stress. Thermal comfort was established using a 7-point scale (where -3 = “much too cool”, 0 = “comfortable” and 3 = “much too warm”; Bedford, 1936) and TS using a 9-point scale (where -4 = “very cold”, 0 = “neutral” and 4 = “very hot”; Zhang et al., 2004). Perceptual data (TC and TS) and blood pressure were recorded at baseline and then at 10 min intervals, beginning 5 min into the trial (Figure 8.3). Resting brachial artery diameter and blood flow velocity were recorded for 1 min at the beginning (10 & 20 min), middle (40 & 50 min) and end (70, & 80 min) stages of the trial. At 21, 51 and 81 min the participants performed the IHG exercise at 50% MVC for 60 s. A visual feedback system was used to ensure the participant produced the desired force during the IHG. Measurement of brachial artery diameter and blood flow velocity were recorded pre (1 min), during and 2 min post-IHG to capture resting artery diameter (as detailed above), as well as the hyperaemic response to the partial occlusion of the brachial artery during the isometric exercise (Figure 8.3; Kagaya & Homma, 1997; Korkmaz & Onalan, 2008; McNeil et al., 2015).

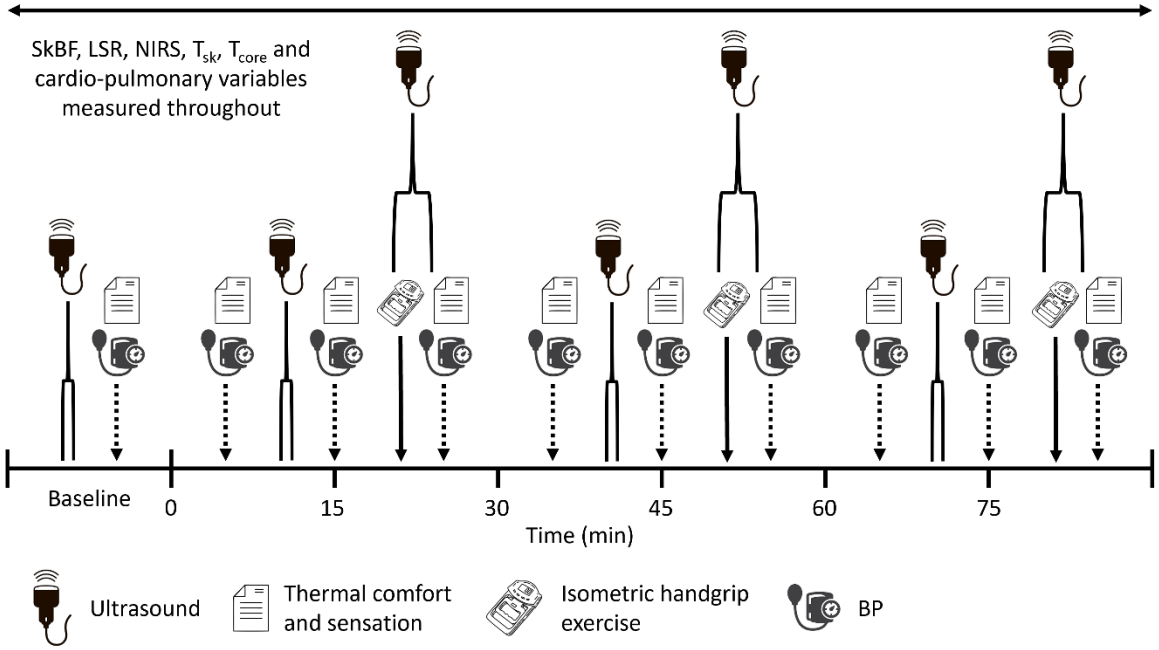


Figure 8.3. Schematic of the trial timeline. *SkBF skin blood flow, LSR local sweat rate, NIRS near infrared spectroscopy, T_{sk} skin temperature, T_{core} core temperature.*

8.3.3.3. Sweat measurements

Participants' body mass was measured pre- and post-trial in thermoneutral conditions outside of the environmental chamber for the measurement of WBSL. This was recorded whilst wearing running shorts, a sports bra for female participants and with the inserted rectal thermistor. A force plate (Type 1758A10, Kistler Instruments Ltd, Farnborough, UK) was used at sampling frequency 1,000 Hz which is highly repeatable within participants, with a test-retest CV of 0.05% for body mass measurements.

Local sweat rate was measured using a Q-sweat system (WR Medical Electronics Co., Stillwater, MN). Compressed dry air was continuously delivered through Teflon-lined tygon tubing at a fixed flow rate of 60 sccm/min to each of the three separate ventilated capsules. Fluid released from the sweat glands was transiently incorporated into the dry air with the change in temperature and RH% monitored by the Q-sweat device. Sweat rate was calculated using standard vapour pressure equations and expressed in nL/min. The sweat capsule size was 0.781 cm^2.

8.3.3.4. Vascular function measurements

The NIRS unit emitted NIR signals at 760 and 850 nm wavelengths to source detectors spaced at 30, 35 and 40 mm. Data were continuously recorded using OxySoft software (Artinis medical systems, Amsterdam, Netherlands) at a sampling frequency of 10 Hz. Relative changes in concentrations of deoxyhaemoglobin ([HHB]), total haemoglobin ([tHB]) and estimated tissue saturation index (TSI%) were recorded during the IHG. Analyses were performed on data acquired from transmitter two (Tx2), rather than Tx1 or Tx3. Tx2 was chosen because its maximum penetrative depth of 17.5 mm incorporated the entire brachioradialis muscle, while limiting the risk of 'noise' from deeper tissues or an overexpression of soft tissue skin and subcutaneous fat (Van Beekvelt et al., 2001).

Video recordings of the brachial artery were analysed using automated edge detection software (FMD, Cardiovascular Suite, Quipu, Italy). The vasodilation function was used to analyse the baseline and resting 1 min recordings to establish resting brachial artery diameter and blood flow velocity. The FMD function was used to analyse the IHG recordings to establish arterial vasodilation% as determined by equation 28:

Arterial vasodilation% = ($[D_{max} - D_{baseline}]/ D_{baseline}$) × 100 (equation 31)

Where D_{max} (mm) is the maximum artery diameter achieved post-IHG and $D_{baseline}$ (mm) is the resting diameter pre-IHG

Shear rate (1/s) was calculated from the Doppler flow velocity waveform (equation 32)

$\text{Shear rate} = (4 \times V_{\text{mean}})/\text{D}\ [1/\text{s}]$ (equation 29)

Where V_{mean} is the time-averaged mean velocity of the blood expressed as cm/s and D (mm) is the artery diameter.

Blood flow was calculated from artery diameter and blood flow velocity at all resting timepoints (equation 33).

$\text{Blood flow} = (V_{\text{mean}}) \times \pi + \left(\frac{\text{D}}{2}\right) 2 \times 60\ [\text{mL/min}]$ (equation 30)

Where π is a mathematical constant, D is the diameter of the artery in cm, and 60 is a constant employed to convert the units to mL/min.

8.3.3.5. Supplementation

After randomisation to the placebo or experimental condition, all supplements were administered in powder form within gelatine capsules, in a double-blind manner. The capsules were matched in number between conditions and contained either 100% isolated taurine or placebo (100% maltodextrin) and were prepared using an analytical balance (Ohaus, Navigator N24120, Nänikon Switzerland). Participants ingested the supplements for a total of eight-days, having 50 mg/kg of body mass per day of taurine or 30 mg/kg of maltodextrin per day across the eight-day period. On day 7 of supplementation, a venous blood sample was taken 1.5 h after the penultimate supplement ingestion. On day 8 of supplementation, the participants undertook the experimental trial and ingested the supplements 30 min before entering the environmental chamber and 1 h prior to trial commencement. The taurine dosage was informed by published recommendations (Waldron et al., 2018a; Warnock et al., 2017) and the timing of ingestion was designed to elicit peak plasma taurine availability during the blood sample and the experimental trial (Ghandforoush-Sattari et al., 2010). Both taurine and maltodextrin were sourced from Myprotein (Manchester, UK).

8.3.3.6. Blood sampling

During visits 2 and 4, venous blood samples were taken in a cool room (~20°C) for the measurement of plasma taurine concentrations. Venous blood samples were obtained via venipuncture from an antecubital vein and were drawn into an EDTA treated vacutainer tubes (6 mL). The tube was immediately placed on ice for 30 min before being centrifuged at 3,000 rev/min for 10 min at 4°C. The plasma was pipetted into 1.5 mL eppendorfs and stored in a -80°C freezer for subsequent analysis of taurine concentrations.

8.3.4. Blood analysis

Plasma taurine concentration was analysed using high performance liquid chromatography with tandem mass spectrometry (HPLC-MS), utilising an Agilent 1290 Infinity II UPLC system alongside a Thermo LTQ Orbitrap XL mass spectrometer. 200 µL of acetonitrile was added to each 100 µL plasma sample in order to precipitate protein, alongside 10 µL of $^{13}C_3$ Alanine as the internal standard. This sample was vortex mixed for 2 min before being centrifuged at 12000 rpm for 10 min. Following centrifugation, 250 µL of the supernatant was diluted with 750 µL of acetonitrile:water (4:1 v/v, with 0.03% formic acid) and this diluted sample was vortex mixed for 30 s. 5 µL of the sample was injected onto a Fortis HILIC HPLC column (30 µm, 2.1 mm × 150 mm) at a flow rate of 200 µL min^{-1} with a mobile phase of acetonitrile:water (19:1

v/v, with 0.03% formic acid). This mobile phase composition was held for 2 min before being increased to 50% water (with 0.03% formic acid) over 8 min and this new mobile phase being held for 2 minutes before returning to the original mobile phase composition and equilibrating the column for a further 10 min. The eluent was analysed by mass spectrometry in negative ionisation mode with a spray voltage of -2.5 kV, sheath gas flow of 20 arbitrary units and a capillary temperature of 350 °C. The mass spectrometer recorded a full scan in Fourier transform (FT) mode (60,000 resolution), followed by a selective ion monitoring (SIM) analysis of the internal standard ion at *m/z* 90 to 92 and a selected reaction monitoring (SRM) analysis of taurine monitoring the transition from *m/z* 124-80. A standard curve was prepared after spiking into bovine plasma and this was used for the quantitation of taurine from the samples analysed, utilising the SIM and SRM peak areas.

8.3.5. Data analysis

Expired gas analysis and cardiovascular variables were reported every 15 min. Skin temperature (mean, calf and thigh), T_{core}, SkBF and sweat rate (chest, thigh, calf and mean [calculated by averaging the three sweat sites]) were reported at baseline and every 15 min. Sweat rate and SkBF onset were determined using segmental linear regression of the variable (LSR or SkBF) – time relationship. Non-linear regression (one-phase association) was used to characterise (tau [time constant] and amplitude) the SkBF, TSI%, [HHB] and [tHB] recovery curve after the IHG to baseline (Graphpad Prism, version 5.01, La Jolla, CA). Due to technical problems, seven participants data were excluded from this analysis. The SkBF response to IHG was characterised using equation 28, where D_{max} is the maximum SkBF value during the IHG and $D_{baseline}$ is the average SkBF value in the preceding minute (Figure 8.8).

8.3.6. Statistical analysis

A two-way RM-ANOVA was conducted with time (baseline and 6 x 15 min stages or IHG 1, 2 and 3) and condition (taurine and placebo) as the independent variables (mean, leg, arm and chest LSR; mean, thigh and calf T_{sk}; T_{core}, TC, TS, SkBF, CVC, MAP, minute ventilation [$\dot{V}E$], $\dot{V}O_2$, $\dot{V}CO_2$, RER, $\dot{Q}$, SV, HR and SPO_2; SkBF tau and amplitude; TSI tau and amplitude; [HHB] tau and amplitude; [tHB] tau and amplitude; SkBF vasodilation%, brachial artery vasodilation%, brachial artery diameter and blood flow). A Greenhouse-Geisser correction was applied when the assumption of sphericity was violated. *Post-hoc* analysis was conducted with Bonferroni correction to identify significant pairwise comparisons if significant interaction effects were observed. Data were checked for normality using the Shapiro-Wilk test (Shapiro

& Wilk, 1965). Two-tailed paired samples *t*-tests were used to identify significant differences between trials at the same comparable timepoints (WBSL, SkBF onset, SkBF thermosensitivity, mean and arm LSR onset, plasma [tau] concentration). A Wilcoxon Signed-Rank test was performed on non-parametric data (chest and leg LSR onset and mean arm chest and leg LSR thermosensitivity). All statistical analysis was conducted in SPSS (IBM SPSS Statistics for Windows, IBM Corp, Version 24.0. Armonk, New York). Data are expressed as means ± SD throughout and a significance level of $p < 0.05$ was accepted across all tests. The magnitude of effects was calculated using Cohen's *d* and partial eta squared (η_p^2) using the following respective criteria of 0.2 and 0.02 (small effect); 0.5 and 0.13 (medium effect); and 0.8 and 0.26 (large effect; Cohen, 1988).

8.4. Results

8.4.1. Plasma taurine concentration

Plasma taurine concentration was greater in the taurine condition *vs* placebo condition (258 ± 55 μM *vs* 74 ± 26 μM; $t_{(12)} = 10.321$, $p < 0.001$, Cohen's $d = 2.863$; Figure 8.4).

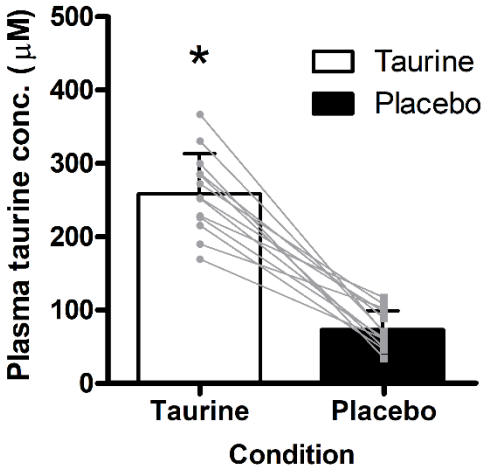


Figure 8.4. Plasma taurine concentrations. *Significantly different than placebo ($p < 0.05$).

8.4.2. Core and skin temperature responses

Core temperature increased with time across both conditions ($F_{(1.219, 14.631)} = 71.726$, $p < 0.001$, $\eta_p^2 = 0.857$); however, there was no main effect of condition ($F_{(1, 12)} = 0.415$, $p = 0.532$, $\eta_p^2 = 0.033$) or an interaction between condition and time ($F_{(1.535, 18.420)} = 0.749$, $p = 0.453$, $\eta_p^2 = 0.059$). Mean, thigh and calf T_{sk} increased with time in both conditions ($F_{(1.283, 15.395)} = 780.189$,

$p < 0.001$, $\eta_p^2 = 0.985$; $F_{(1.552, 18.623)} = 282.758$, $p < 0.001$, $\eta_p^2 = 0.959$; $F_{(1.720, 20.639)} = 432.219$, $p < 0.001$, $\eta_p^2 = 0.973$, respectively), however, there was no main effect of condition ($F_{(1, 12)} = 0.045$, $p = 0.835$, $\eta_p^2 = 0.004$; $F_{(1, 12)} = 0.961$, $p = 0.346$, $\eta_p^2 = 0.074$; $F_{(1, 12)} = 0.406$, $p = 0.536$, $\eta_p^2 = 0.033$, respectively) or an interaction effect with time ($F_{(1.506, 18.068)} = 0.273$, $p = 0.702$, $\eta_p^2 = 0.022$; $F_{(1.961, 23.536)} = 1.171$, $p = 0.327$, $\eta_p^2 = 0.089$; $F_{(2.168, 26.012)} = 0.687$, $p = 0.523$, $\eta_p^2 = 0.054$, respectively; Figure 8.5).

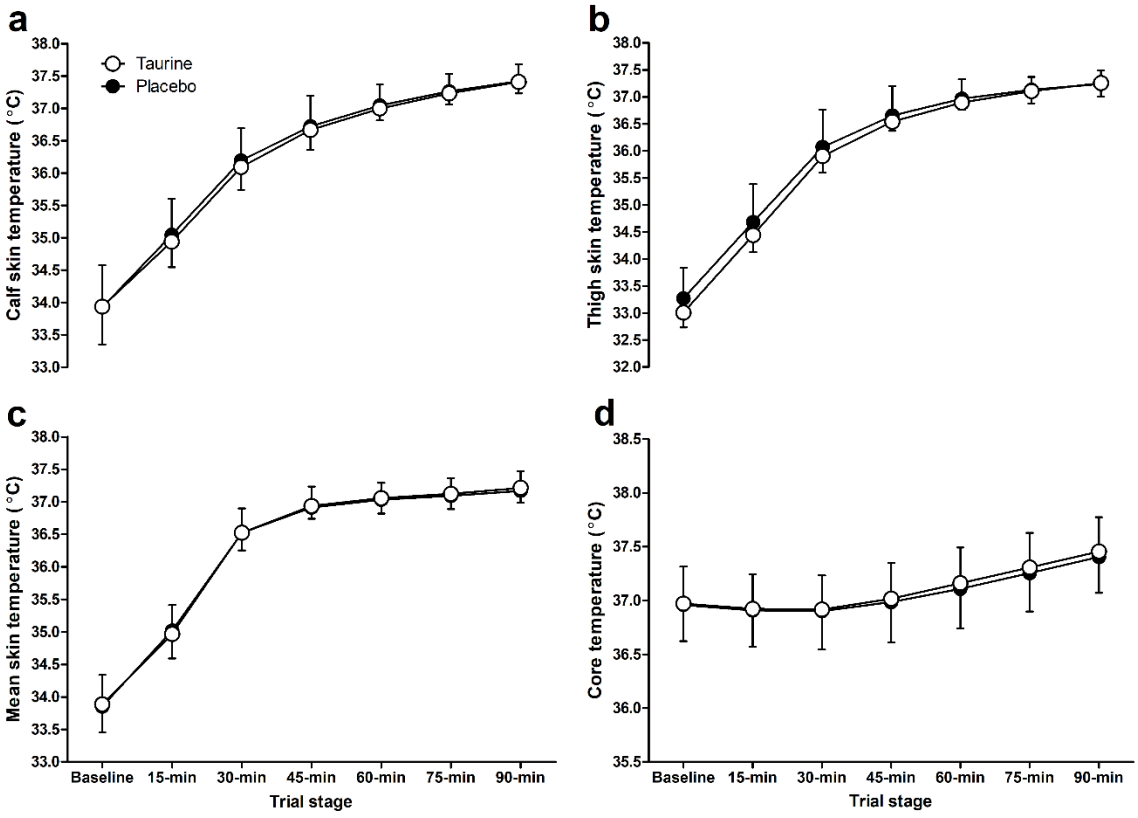


Figure 8.5. Calf (a), thigh (b) and mean (c) skin temperature and core temperature (d) plotted across time in the experimental trials (mean ± SD).

8.4.3. Sweating measurements

Local sweat rate variables are displayed in Figure 8.6. Whole-body sweat loss (Taurine = 478 ± 177 mL; Placebo = 411 ± 174 mL; $t_{(12)} = 2.179$, $p = 0.049$, Cohen's $d = 0.380$) and leg LSR ($F_{(1, 12)} = 8.951$, $p = 0.011$, $\eta_p^2 = 0.427$; Figure 8.7) were greater in the taurine condition compared to placebo. However, there was no difference in mean LSR ($F_{(1, 12)} = 0.380$, $p = 0.549$, $\eta_p^2 = 0.031$), arm LSR ($F_{(1, 12)} = 0.562$, $p = 0.468$, $\eta_p^2 = 0.045$) and chest LSR ($F_{(1, 12)} = 2.350$, $p = 0.151$, $\eta_p^2 = 0.164$) between conditions. There was a time effect for leg LSR, mean LSR, arm LSR and chest LSR ($F_{(1.766, 21.195)} = 46.335$, $p < 0.001$, $\eta_p^2 = 0.794$; $F_{(1.261, 15.133)} = 63.133$, $p < 0.001$, $\eta_p^2 = 0.842$; $F_{(1.305, 15.665)} = 44.366$, $p < 0.001$, $\eta_p^2 = 0.787$; $F_{(1.142, 13.699)} =$

51.039, $p < 0.001$, $\eta_p^2 = 0.810$, respectively) and a condition x time interaction effect for leg LSR ($F_{(1.508, 18.097)} = 5.279$, $p = 0.022$, $\eta_p^2 = 0.306$), though not for mean LSR ($F_{(2.246, 26.952)} = 1.165$, $p = 0.331$, $\eta_p^2 = 0.089$), arm LSR ($F_{(1.706, 20.473)} = 1.084$, $p = 0.347$, $\eta_p^2 = 0.083$) and chest LSR ($F_{(2.100, 25.200)} = 1.644$, $p = 0.213$, $\eta_p^2 = 0.120$). Post-hoc pairwise analysis revealed that leg LSR was only greater in the taurine condition at 60-, 75- and 90 min (277 ± 120 *vs* 211 ± 92 nL/min, $p = 0.011$; 309 ± 122 *vs* 225 ± 108 nL/min, $p = 0.010$; 335 ± 136 *vs* 244 ± 130 nL/min, $p = 0.022$, respectively). Mean ($t_{(12)} = -1.563$, $p = 0.144$, Cohen's $d = -0.343$; Z = -1.494, $p = 0.135$, Cohen's $d = -0.414$), arm ($t_{(12)} = -1.153$, $p = 0.271$, Cohen's $d = -0.295$; Z = 0.245, $p = 0.807$, Cohen's $d = 0.068$), chest (Z = -0.874, $p = 0.382$, Cohen's $d = -0.242$; Z = -1.434, $p = 0.151$, Cohen's $d = -0.398$) and leg (Z = 0.384, $p = 0.701$, Cohen's $d = 0.107$; Z = -0.878, $p = 0.380$, Cohen's $d = -0.244$) sweat onset times and thermosensitivity, respectively, were not different between conditions.

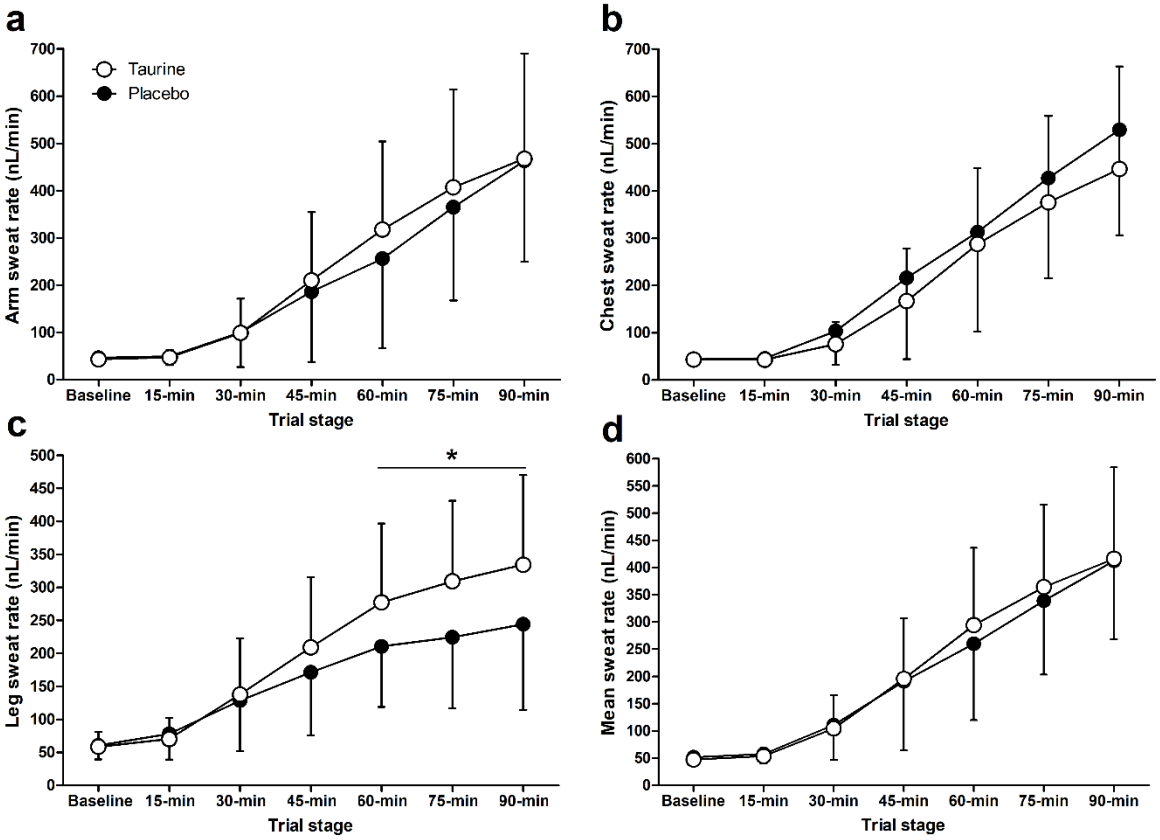


Figure 8.6. Arm (a), chest (b), leg (c) and mean (d) sweat rate plotted across time in the experimental trials (mean ± SD). *Significantly greater than placebo ($p < 0.05$).

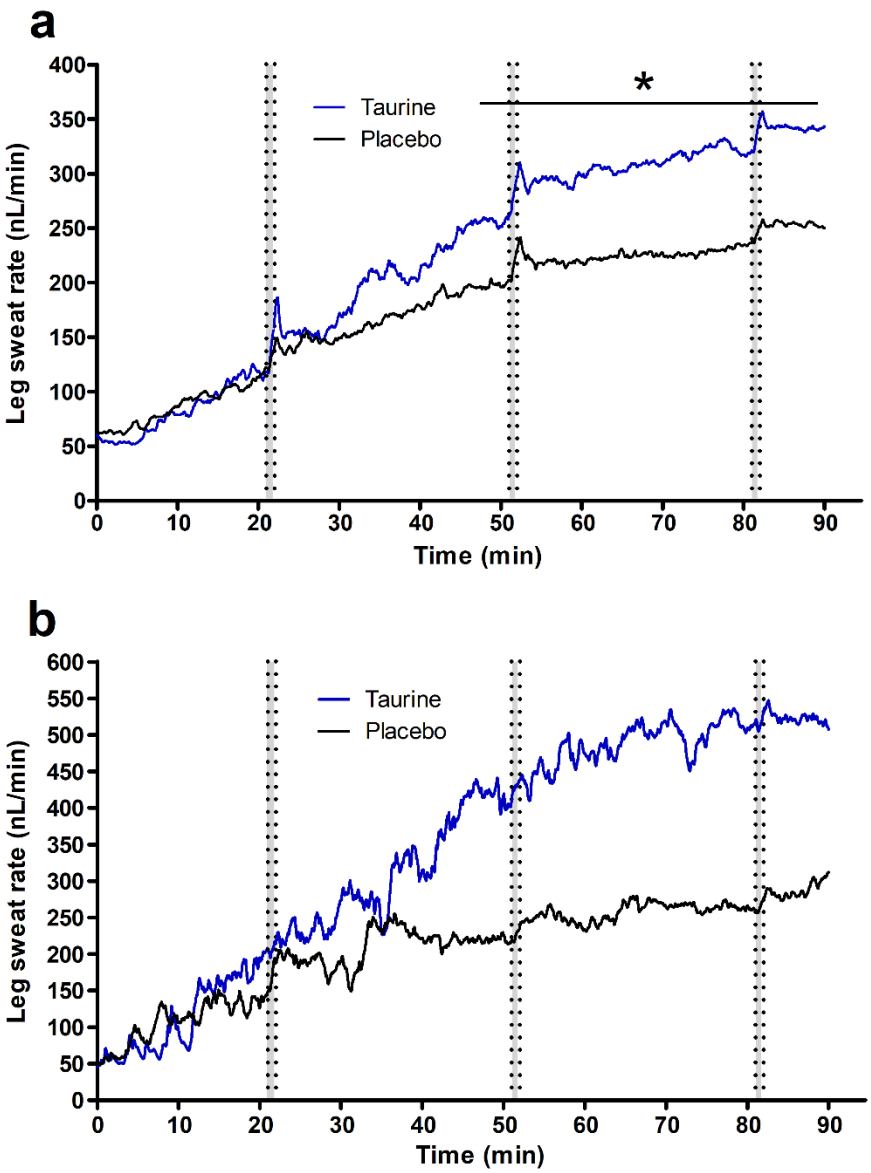


Figure 8.7. Mean leg sweat rate trace (a) and example leg sweat rate time series of a representative participant during the experimental trial (b). Vertical grey bars = isometric handgrip exercise. *Significantly greater than placebo (p < 0.05).

8.4.4. Vascular function measurements

Skin blood flow and CVC were greater in the taurine condition compared to placebo ($F_{(1, 12)}$ = 7.859, p = 0.016, η_p^2 = 0.396; $F_{(1, 12)}$ = 6.383, p = 0.027, η_p^2 = 0.347, respectively; Figure 8.9). There was a time effect for normalised SkBF ($F_{(2.146, 25.757)}$ = 64.933, p < 0.001, η_p^2 = 0.844) and CVC ($F_{(2.158, 25.900)}$ = 63.770, p < 0.001, η_p^2 = 0.842), but no interaction effect ($F_{(1.615, 19.385)}$ = 2.626, p = 0.107, η_p^2 = 0.180; $F_{(1.609, 19.310)}$ = 2.276, p = 0.137, η_p^2 = 0.159, respectively). Skin blood flow onset appeared earlier in the taurine condition compared to placebo ($t_{(12)}$ = -2.706,

p = 0.019, Cohen's d = 0.751), but there was no difference in thermosensitivity ($t_{(12)}$ = 1.222, p = 0.245, Cohen's d = 0.068). SkBF ($F_{(1, 12)}$ = 7.163, p = 0.020, η_p^2 = 0.374) and brachial artery vasodilation% ($F_{(1, 12)}$ = 15.017, p = 0.002, η_p^2 = 0.556), during and post the IHG and the tau ($F_{(1, 5)}$ = 7.824, p = 0.038, η_p^2 = 0.610) and amplitude ($F_{(1, 5)}$ = 10.760, p = 0.022, η_p^2 = 0.683) of the SkBF recovery to baseline were greater in taurine *vs* placebo. There was an increase with time for SkBF ($F_{(2, 24)}$ = 7.536, p = 0.003, η_p^2 = 0.386) and brachial artery vasodilation% ($F_{(2, 24)}$ = 28.619, p < 0.001, η_p^2 = 0.705); however, no time effect for the tau ($F_{(1.068, 5.338)}$ = 1.649, p = 0.255, η_p^2 = 0.248) and amplitude ($F_{(2, 10)}$ = 3.068, p = 0.091, η_p^2 = 0.380) of the IHG recovery and no time x condition interaction effect for any variable, respectively ($F_{(1.167, 13.998)}$ = 2.481, p = 0.135, η_p^2 = 0.171; $F_{(2, 24)}$ = 1.274, p = 0.298, η_p^2 = 0.096; $F_{(1.060, 5.298)}$ = 2.291, p = 0.188, η_p^2 = 0.314; $F_{(2, 10)}$ = 2.511, p = 0.131, η_p^2 = 0.334; Table 8.1). A representative trace of SkBF is provided to visually display the analyses of these variables (Figure 8.8). Mean arterial pressure, brachial artery diameter and blood flow increased with time in both conditions ($F_{(9, 108)}$ = 4.112, p < 0.001, η_p^2 = 0.255; $F_{(2.638, 31.656)}$ = 48.416, p < 0.001, η_p^2 = 0.801; $F_{(6, 72)}$ = 55.087, p < 0.001, η_p^2 = 0.821); however, there was no condition effect ($F_{(1, 12)}$ = 0.516, p = 0.486, η_p^2 = 0.041; $F_{(1, 12)}$ = 4.376, p = 0.058, η_p^2 = 0.267; $F_{(1, 12)}$ = 0.411, p = 0.533, η_p^2 = 0.033) or time x condition interaction effect for any variable, respectively ($F_{(9, 108)}$ = 0.989, p = 0.453, η_p^2 = 0.076; $F_{(6, 72)}$ = 1.493, p = 0.193, η_p^2 = 0.111; $F_{(6, 72)}$ = 1.108, p = 0.366, η_p^2 = 0.085; Figure 8.10). There was no difference in shear rate across time ($F_{(2, 24)}$ = 1.572, p = 0.228, η_p^2 = 0.300) or condition ($F_{(1, 12)}$ = 3.184, p = 0.100, η_p^2 = 0.375) and no time x condition interaction effect ($F_{(2, 24)}$ = 0.728, p = 0.493, η_p^2 = 0.159).

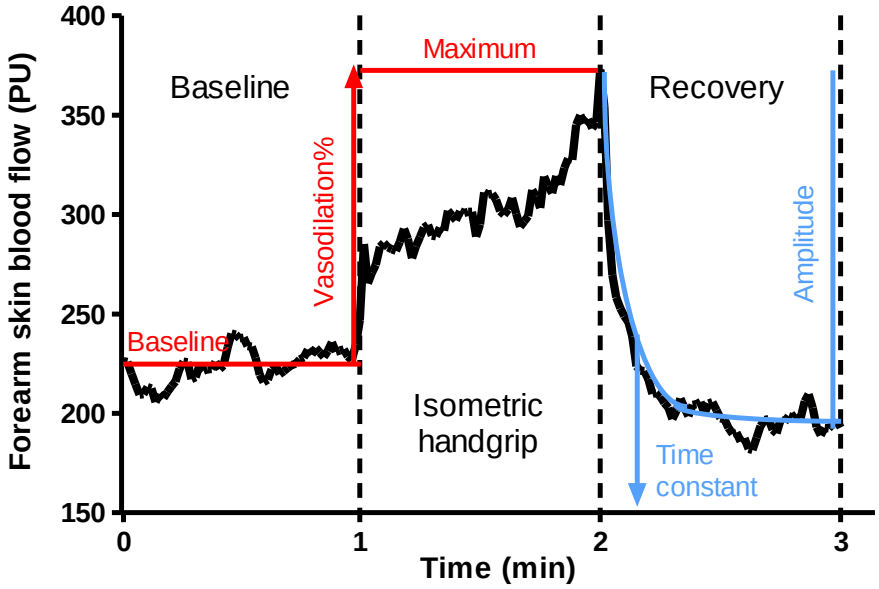


Figure 8.8. Example forearm skin blood flow time series of a representative participant pre, during and post isometric handgrip exercise, displaying the variables used to calculate Vasodilation% (red) and the one-way association recovery curve with the amplitude and time constant (blue).

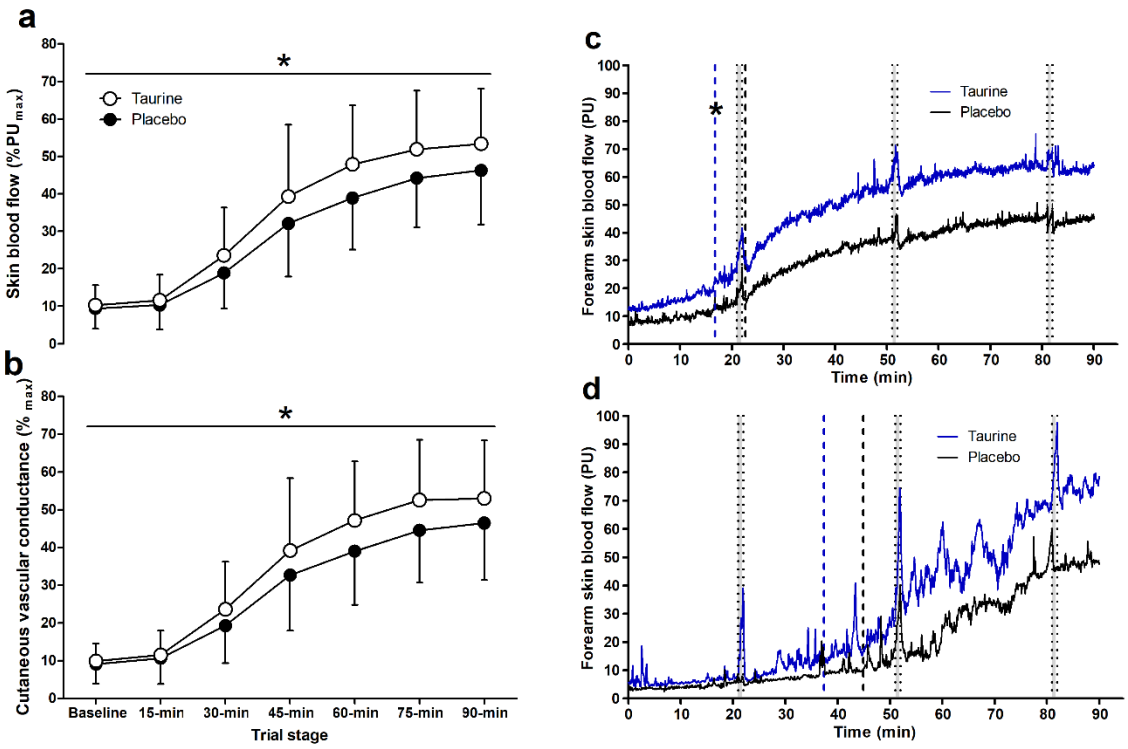


Figure 8.9. Skin blood flow (a) and cutaneous vascular conductance (b), plotted across time in the experimental trials (mean ± SD). Mean skin blood flow trace (c) and example skin blood

flow time series of a representative participant during the experimental trial (d). Blue (taurine) and black (placebo) dashed lines = skin blood flow onset point, grey bars = isometric handgrip exercise. *Significantly greater than placebo ($p < 0.05$).

Table 8.1. Skin blood flow vasodilation% during isometric handgrip exercise, and brachial artery vasodilation% and shear rate post-isometric handgrip exercise (mean and SD).

	Taurine	Placebo	Taurine	Placebo	Taurine	Placebo
Variable	IHG 1		IHG 2		IHG 3	
Skin vasodilation%	189 ± 200	88 ± 61	92 ± 93	65 ± 76	46 ± 52	28 ± 22
Artery vasodilation%	10.2 ± 3.2	8.9 ± 2.9	6.9 ± 2.9	3.9 ± 2.0	7.0 ± 2.5	4.5 ± 1.5
Shear rate (1/s)	1147 ± 243	1244 ± 350	1106 ± 239	1204 ± 330	1099 ± 194	1284 ± 373

Note: IHG isometric handgrip.

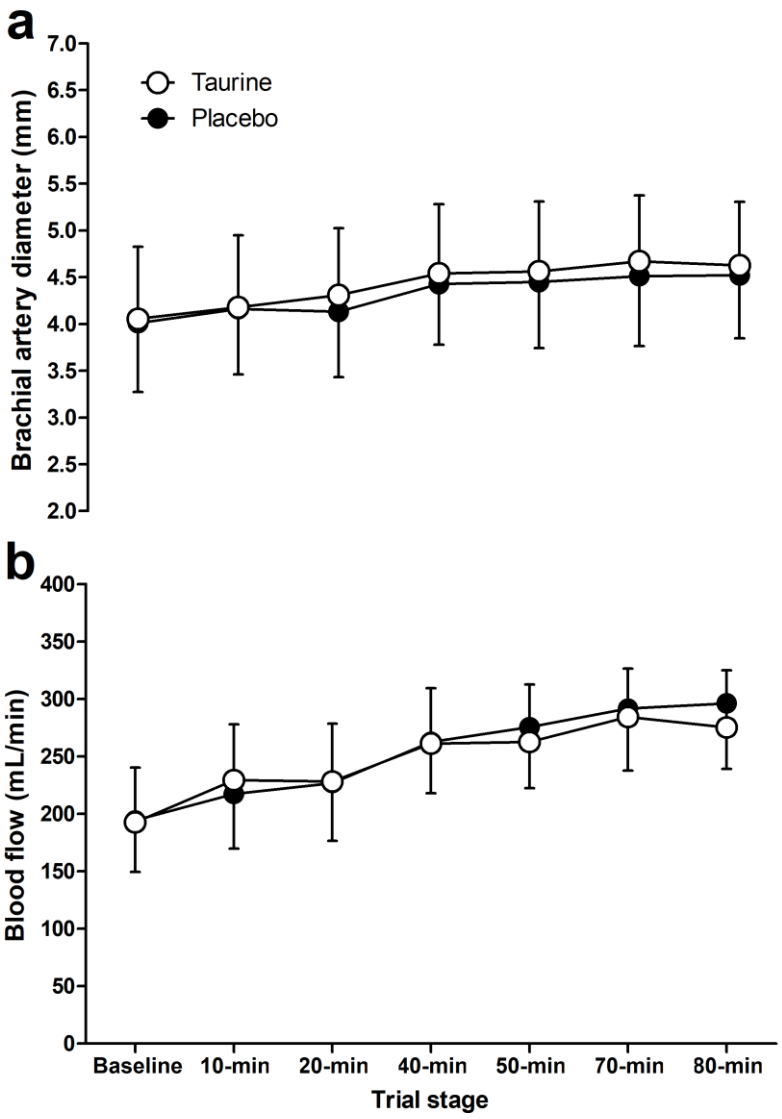


Figure 8.10. Resting brachial artery diameter (a) and blood flow (b), plotted across time in the experimental trials (mean ± SD).

8.4.5. Metabolic function measurements

All muscle oxygenation variables are displayed in Table 8.2. There was no effect of time on TSI tau, TSI amplitude, [HHB] tau, [HHB] amplitude or [tHB] amplitude ($F_{(2, 8)}$ = 2.983, p = 0.108, η_p^2 = 0.427; $F_{(1, 4)}$ = 2.076, p = 0.188, η_p^2 = 0.342; $F_{(1.073, 5.367)}$ = 0.235, p = 0.664, η_p^2 = 0.045; $F_{(1.101, 5.507)}$ = 1.667, p = 0.252, η_p^2 = 0.250; $F_{(1.025, 5.123)}$ = 3.203, p = 0.132, η_p^2 = 0.390, respectively), however there was for [tHB] tau ($F_{(2, 10)}$ = 7.373, p = 0.011, η_p^2 = 0.596). There was no effect of condition for TSI tau, TSI amplitude, [HHB] tau, [HHB] amplitude, [tHB] tau or

[tHB] amplitude ($F_{(1, 4)}$ = 1.437, p = 0.297, η_p^2 = 0.264; $F_{(1, 4)}$ = 0.340, p = 0.591, η_p^2 = 0.078; $F_{(1, 5)}$ = 0.837, p = 0.402, η_p^2 = 0.143; $F_{(1, 5)}$ = 0.068, p = 0.804, η_p^2 = 0.013; $F_{(1, 5)}$ = 0.914, p = 0.383, η_p^2 = 0.155; $F_{(1, 5)}$ = 0.039, p = 0.850, η_p^2 = 0.008, respectively) and or condition x time interaction effect ($F_{(2, 8)}$ = 0.292, p = 0.754, η_p^2 = 0.068; $F_{(2, 8)}$ = 0.277, p = 0.765, η_p^2 = 0.065; $F_{(2, 10)}$ = 1.126, p = 0.362, η_p^2 = 0.184; $F_{(1{,}125, 5.625)}$ = 0.697, p = 0.455, η_p^2 = 0.122; $F_{(2, 10)}$ = 1.767, p = 0.220, η_p^2 = 0.261; $F_{(2, 10)}$ = 0.313, p = 0.738, η_p^2 = 0.059, respectively).

Table 8.2. Skin blood flow and muscle oxygenation recovery post-isometric handgrip exercise.

		Taurine	Placebo	Taurine	Placebo	Taurine	Placebo
Variable		IHG 1		IHG 2		IHG 3	
Skin blood flow	Time constant	8.4 ± 3.2	3.7 ± 2.8	6.4 ± 3.7	6.8 ± 5.8	4.2 ± 2.3	3.3 ± 1.1
(*n* = 6)	Amplitude	-80.7 ± 40.6	-32.7 ± 16.8	-116.0 ± 59.8	-59.0 ± 36.2	-68.41 ± 54.1	-68.0 ± 37.6
Tissue saturation	Time constant	7.4 ± 5.9	6.9 ± 4.5	5.0 ± 4.3	3.8 ± 1.4	1.9 ± 1.0	1.7 ± 0.7
index % (*n* = 5)	Amplitude	12.7 ± 5.9	14.6 ± 6.7	9.1 ± 3.3	9.2 ± 2.2	8.9 ± 5.5	9.5 ± 4.8
Deoxy-haemoglobin	Time constant	18.3 ± 23.6	13.0 ± 10.4	15.3 ± 17.8	19.9 ± 25.8	17.3 ± 24.0	8.3 ± 5.1
(*n* = 6)	Amplitude	-9.7 ± 5.3	-10.7 ± 7.0	-8.6 ± 4.9	-10.5 ± 10.3	-20.6 ± 20.9	-14.2 ± 13.8
Total haemoglobin	Time constant	0.8 ± 0.2	1.1 ± 0.4	1.0 ± 0.4	0.9 ± 0.3	0.5 ± 0.3	0.6 ± 0.5
(*n* = 6)	Amplitude	13.8 ± 11.0	14.1 ± 9.8	18.7 ± 13.9	17.3 ± 12.2	25.2 ± 25.0	27.1 ± 21.3

Note: IHG isometric handgrip.

8.4.6. Cardio-pulmonary measurements

All pulmonary variables are displayed in Table 8.3 and cardiovascular variables displayed in Figure 8.11. There was a time effect for $\dot{V}E$, $\dot{V}O_2$, $\dot{V}CO_2$, RER, $\dot{Q}$, SV and HR ($F_{(1.983, 23.794)}$ = 16.716, $p < 0.001$, η_p^2 = 0.582; $F_{(5, 60)}$ = 29.068, $p < 0.001$, η_p^2 = 0.708; $F_{(2.505, 30.066)}$ = 20.477, $p < 0.001$, η_p^2 = 0.631; $F_{(2.536, 30.430)}$ = 6.582, p = 0.002, η_p^2 = 0.927; $F_{(5, 60)}$ = 12.897, $p < 0.001$, η_p^2 = 0.518; $F_{(2.748, 32.978)}$ = 53.721, $p < 0.001$, η_p^2 = 0.817; $F_{(2.459, 29.511)}$ = 173.065, $p < 0.001$, η_p^2 = 0.935, respectively), but not for SPO_2 ($F_{(2.074, 24.883)}$ = 1.444, p = 0.255, η_p^2 = 0.107). There was no condition effect for $\dot{V}E$, $\dot{V}O_2$, $\dot{V}CO_2$, RER, $\dot{Q}$, SV, HR and SPO_2 ($F_{(1, 12)}$ = 0.290, p = 0.600, η_p^2 = 0.024; $F_{(1, 12)}$ = 0.743, p = 0.406, η_p^2 = 0.058; $F_{(1, 12)}$ = 0.355, p = 0.562, η_p^2 = 0.029; $F_{(1, 12)}$ = 0.077, p = 0.787, η_p^2 = 0.057; $F_{(1, 12)}$ = 0.466, p = 0.508, η_p^2 = 0.037; $F_{(1, 12)}$ = 1.384, p = 0.262, η_p^2 = 0.103; $F_{(1, 12)}$ = 0.020, p = 0.890, η_p^2 = 0.002; $F_{(1, 12)}$ = 0.043, p = 0.840, η_p^2 = 0.004, respectively) and also no condition x time interaction effect ($F_{(2.368, 28.421)}$ = 0.885, p = 0.439, η_p^2 = 0.069; $F_{(2.639, 31.666)}$ = 1.660, p = 0.200, η_p^2 = 0.122; $F_{(5, 60)}$ = 2.246, p = 0.061, η_p^2 = 0.158; $F_{(2.910, 34.923)}$ = 0.404, p = 0.745, η_p^2 = 0.121; $F_{(2.055, 24.664)}$ = 1.660, p = 0.615, η_p^2 = 0.040; $F_{(1.910, 22.917)}$ = 0.371, p = 0.685, η_p^2 = 0.030; $F_{(2.372, 28.469)}$ = 1.791, p = 0.180, η_p^2 = 0.130; $F_{(2.317, 27.809)}$ = 0.392, p = 0.709, η_p^2 = 0.032, respectively).

Table 8.3. Minute ventilation, oxygen uptake and carbon dioxide production across time in the experimental trials (mean ± SD).

Variable		15 min	30 min	45 min	60 min	75 min	90 min	Mean
Minute ventilation (L/min)	Taurine	7.9 ± 0.8	8.4 ± 0.9	8.0 ± 1.0	8.7 ± 1.0	8.2 ± 0.8	9.0 ± 1.2	8.4 ± 0.9
	Placebo	7.8 ± 1.3	8.3 ± 1.2	8.1 ± 1.1	8.7 ± 1.0	8.5 ± 1.2	9.2 ± 1.5	8.4 ± 1.1
Oxygen uptake (mL/min)	Taurine	311.1 ± 45.5	322.8 ± 51.1	322.8 ± 52.4	340.3 ± 48.3	323.0 ± 59.0	348.0 ± 62.2	329.1 ± 51.3
	Placebo	310.3 ± 55.1	319.0 ± 45.7	324.8 ± 50.4	343.4 ± 45.5	346.6 ± 41.5	359.7 ± 43.3	334.0 ± 45.4
Carbon dioxide production (mL/min)	Taurine	253.1 ± 35.6	279.2 ± 45.8	262.3 ± 42.1	288.8 ± 40.4	268.2 ± 39.1	297.7 ± 56.4	274.9 ± 40.6
	Placebo	253.0 ± 51.1	272.5 ± 44.0	262.9 ± 43.7	290.3 ± 44.7	283.4 ± 40.2	309.2 ± 49.5	278.6 ± 43.0
Respiratory exchange ratio	Taurine	0.81 ± 0.04	0.86 ± 0.04	0.80 ± 0.04	0.84 ± 0.05	0.81 ± 0.04	0.85 ± 0.05	0.83 ± 0.03
	Placebo	0.81 ± 0.05	0.85 ± 0.04	0.80 ± 0.04	0.83 ± 0.04	0.81 ± 0.03	0.85 ± 0.05	0.83 ± 0.03

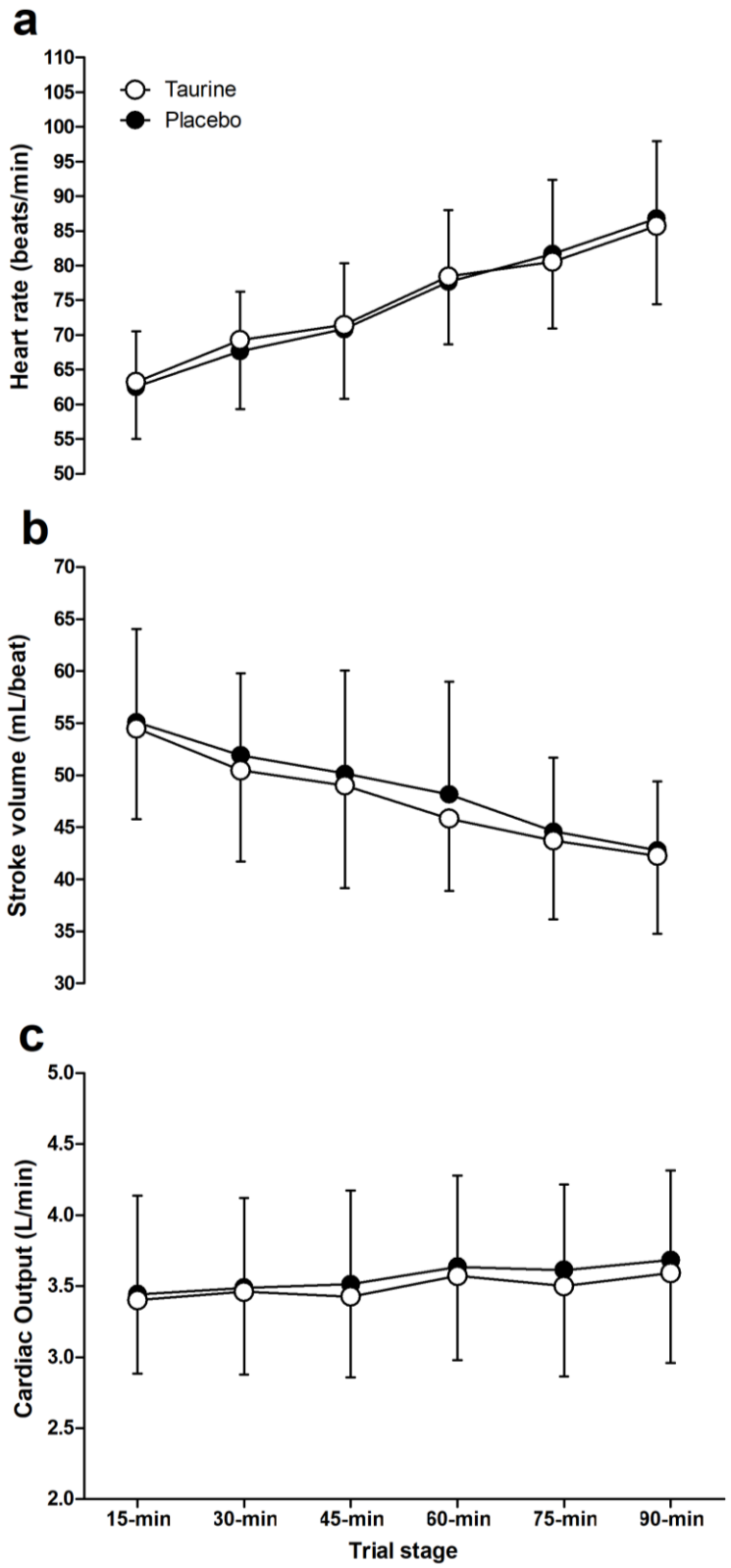


Figure 8.11. Heart rate (a), stroke volume (b) and cardiac output (c) plotted across time in the experimental trials (mean ± SD).

8.4.7. Perceptual measurements

Thermal comfort and TS increased with time in both conditions ($F_{(1.753, 21.032)}$ = 68.196, p < 0.001, η_p^2 = 0.850; $F_{(1.998, 23.980)}$ = 105.787.196, p < 0.001, η_p^2 = 0.898, respectively); however, there was no main effect for condition ($F_{(1, 12)}$ = 0.645, p = 0.437, η_p^2 = 0.051; $F_{(1, 12)}$ = 0.791, p = 0.391, η_p^2 = 0.062, respectively) and no interaction effect ($F_{(1.456, 17.470)}$ = 1.513, p = 0.244, η_p^2 = 0.112; $F_{(3, 36)}$ = 2.447, p = 0.080, η_p^2 = 0.169, respectively).

8.5. Discussion

This study investigated the effect of eight-days oral taurine supplementation on sweating, cardio-pulmonary, metabolic and T_{core} responses during 90 min passive heating in healthy females and males. In acceptance of the hypothesis, some sweating and vascular parameters, such as SkBF (perfusion units, CVC and skin vasodilation%), brachial artery vasodilation%, WBSL and leg LSR, were increased following taurine supplementation. However, contrary to the hypothesis, resting brachial artery diameter, blood flow, blood pressure (MAP), arm and chest LSR were not significantly greater in the taurine condition and there were no observed changes in T_{core} and T_{sk} or cardiovascular parameters ($\dot{Q}$, SV and HR). Therefore, oral taurine supplementation appears to provide thermoregulatory benefits during passive heat exposure, which is primarily related to improved sweating and cutaneous vasodilation.

8.5.1. Sweating responses

There was a 16.2% increase in WBSL across the passive heating protocol in the taurine condition compared to placebo. Two prior studies have also reported a greater sweating response following taurine supplementation in the heat (Page et al., 2019; Chapter Seven) and, therefore, the current study provides supporting evidence that taurine elicits a systemic sweating response. These changes in WBSL, however, are smaller than those reported previously during low-intensity exercise at a fixed $\dot{H}_{prod}$ (~200 W/m^2) and humidity (~26.6%; Chapter Seven) or during cycling at a higher intensity (~72.7%; Page et al., 2019), which is perhaps related to the resting conditions and reduced sweating demand in the current study. There was no difference in mean LSR between conditions, which was unanticipated, considering the current WBSL results and the previous findings of 12.7% (Page et al., 2019) and 15.5% (Chapter Seven) increases during exercise in the heat. However, there is a non-linear relationship between WBSL and LSR (Baker et al., 2018), as LSR is site specific (Smith

& Havenith, 2011). Indeed, in the current study, a significantly greater sweating response was observed at the leg (26.6%), but at no other measured site on the body (arm or chest), following taurine ingestion.

The greater local sweating observed at the leg site can be explained by the methods of the current study. Participant's legs were covered in reflective foil, creating a hot and humid microclimate surrounding the lower limbs. This enclosed area would likely have a high $\dot{E}_{req}$ and low $\dot{E}_{max}$, alongside a negative skin to ambient air temperature gradient. Consequently, there would be limited capacity for evaporative and dry heat transfer, inducing greater local heat strain. Interestingly, this was the only site at which LSR demonstrated an increase following taurine supplementation, suggesting that the effect of taurine may be partially dependent upon heat strain. Further, the magnitude to which taurine enhanced WBSL was substantially lower during the current protocol, compared to previously employed exercise regimes eliciting greater $\dot{H}_{prod}$ and rises in T_{core}, indicative of a larger heat strain (Page et al., 2019; Chapter Seven). Finally, the discrepancy between the taurine-mediated augmentation of leg LSR and the placebo comparison became greater as the trial progressed (Figure 8.6.c Figure 8.8.a). This occurred alongside increased T_{core} and heat storage, as also observed previously (Page et al., 2019). Collectively, this provides preliminary evidence that taurine-induced augmentation of the sweating response may be somewhat heat strain or $\dot{E}_{req}$-mediated. Taurine may, therefore, be most efficacious in scenarios of heightened thermal strain, occurring through either oppressive environmental conditions, which limit heat dissipation, or increased $\dot{H}_{prod}$. Further research establishing the magnitude of augmented sweating in response to taurine supplementation at varying degrees of heat strain would help elucidate this relationship.

The above findings are indicative of taurine inducing a progressive sweating effect, growing as a function of thermal exposure time, at least at the local leg site. These results deviate from those in Chapter Seven, where sweating was greater toward the beginning of the trial and reduced in the latter stages of exercise in the heat. However, in the previous study (Chapter Seven), LSR was not continuously measured across time, as absorbent patches were used to establish LSR, as opposed to the ventilated capsule technique implemented here. Notwithstanding the differences in the measurement techniques used between these studies, the different time-course of responses could also be attributed to the resting and exercising states, with the previous exercising condition providing more substantial demand for sweating in the early parts of the trials, despite this being a relatively lower heat strain than the end of the trial. Furthermore, it is possible that, due to the length of the previous study (~145 min), the effects of taurine were beginning to decline towards the end of the trial, due to a reduction in plasma availability, as taurine has a half-life of 60 min (Ghandforoush-Sattari et al., 2010).

The taurine condition clearly increased local leg sweating across time and the current analysis of the sweating onset time for mean LSR (~22 *vs* ~26 min) and leg LSR (~17 *vs* ~20 min) also demonstrated a tendency for earlier sweating in the taurine condition compared to placebo, however, there was no significant difference between conditions. There was also no greater sweating thermosensitivity at any LSR site, despite this trend towards a shorter onset time. It is recommended that this potential for earlier onset of sweating is considered in future research, since it is a key feature of the acclimated phenotype (Périard et al., 2016) and, therefore, it is important to understand if, under elevated heat strain, taurine may facilitate it. An increased sweating 'sensitivity' (i.e. earlier onset) could have important implications for individuals exposed to moderate levels of thermal stress, including athletes, military personnel, occupational workers and those living in hot climates, offering immediate means of heat transfer. Although, this will be reliant on the capacity to manipulate clothing coverage to facilitate evaporative heat transfer from the skin. Depending on the sport, military or occupational setting, it is unclear whether this would be feasible and provides a limitation to the application of these findings.

Whilst a centrally-mediated mechanism cannot be ruled out, based on the later time-course of the leg sweating responses reported in the current study, it is more likely that a peripheral mechanism is responsible (Ravanelli et al., 2021). As a free amino acid, taurine has an established role as an osmolyte (Cuisinier et al., 2002; Huxtable, 1992), where its concentration in intra- and extra-cellular compartments acts to maintain osmotic pressure (Pasantes-Morales et al., 1998). Further, TauT are abundantly expressed in many tissues, including the skin (Baliou et al., 2020; Da Silva et al., 2008; Han et al., 2006), allowing taurine to move freely and affect fluid regulation throughout the body (Mozaffari & Schaffer, 2001). Theoretically, greater accumulation of taurine in the interstitial fluid, which might occur through dietary supplementation, would increase the osmotic gradient in this compartment, thereby drawing additional fluid into this space and increasing availability to sweat glands. However, two previous studies have shown no relationship between plasma taurine concentrations and plasma volume (Cuisinier et al., 2002; Chapter Seven), though these were inconclusive and fluid accumulation in the extracellular compartment following taurine supplementation in humans is yet to be established. It is possible that skin microdialysis techniques could be used to detect this potential change in future studies.

It is also possible that improved SkBF may provide greater fluid availability to the sweat gland, as an association between SkBF and sweating has been established (Brengelmann et al., 1973; Nadel et al., 1971; Nadel et al., 1971; Van Beaumont & Bullard, 1965). Despite this, there is little evidence of a direct causal relationship, and several studies have demonstrated that increases in SkBF are not a prerequisite for increases in sweat rate (Cramer et al., 2017;

Ravanelli et al., 2017). In the current study, the mean onset of SkBF did precede the mean onset of mean LSR (~20 *vs* ~24 min), suggesting that it may, in part, be associated with this subsequent increase in sweat rate. Furthermore, the onset of SkBF at the arm was reduced in the taurine condition compared to placebo (~17 *vs* ~23 min), which was followed by a trend towards a quicker arm LSR onset time (~25 *vs* ~30 min). However, individually, there was no clear ordering pattern, and there was no difference in the time differential of the onset times between conditions, indicating that these responses may not be coupled. Nevertheless, there appears to be a requirement of SkBF for sustained sweating (Collins et al., 1959; MacIntyre et al., 1968; Wingo et al., 2010), which is somewhat supported by the results herein. Arm LSR measured alongside SkBF, demonstrated a non-significant ~8.5% increase following taurine ingestion, which was parallelled throughout the trial by greater SkBF. However, in the absence of more definitive results through manipulation of sweat onset times, the interplay between these physiological responses and whether this provides mechanistic explanation for the taurine-induced sweating augmentation can only be speculated upon.

8.5.2. Vascular responses

This current study is the first to demonstrate that taurine supplementation during resting (passive) heat exposure improves active cutaneous vasodilation. Both SkBF and CVC were greater in the taurine condition, compared to placebo. This develops upon previous studies, where *in-vitro* studies have repeatedly demonstrated taurine's ability to induce vaso-relaxation in macro-vessels (Yildiz & Ulusoy, 2022), which appears to occur through both endothelium-dependant and independent pathways (Maia et al., 2014; Ulusoy et al., 2017). Several likely mechanisms have been proposed for these findings, including reduction in Ca^{2+} influx and release (Dawson et al., 2000; Franconi et al., 1982; Harada et al., 2004; Li et al., 2009), anti-oxidation (Hagar et al., 2006; Leão et al., 2019; Maia et al., 2014; Rahman et al., 2011), improved NO bioavailability (Maia et al., 2014; Rahman et al., 2011), elevation of plasma H_2S concentrations (Sun et al., 2016) and K^+ channel opening (Liu et al., 2009; Ulusoy et al., 2017). Additionally, vascular smooth muscle within the arterioles of the skin has many TauT (Liao et al., 2007), where taurine may directly affect vascular tone through the aforementioned effects on calcium handling and K^+ channels (Li et al., 1996; Liu et al., 2009; Ristori & Verdetti, 1991; Ulusoy et al., 2017). Some of these mechanisms might also be responsible for the heat induced vasodilation of the subcutaneous vasculature, demonstrated here during rest. Although additional research is required to understand this further, the effect of H_2S on endothelial cell signalling or on vascular smooth muscle function indicates it could potentially be a responsible factor. For example, it has been established, *in-vivo*, that taurine exerts

endothelium-dependant and independent vasodilation in humans with concomitant increases in plasma H_2S concentrations (Sun et al., 2016). Consistent with these findings, in addition to the passive increases in subcutaneous blood flow after taurine supplementation, a greater hyperaemic response in the brachial artery following partial occlusion (induced by IHG exercise) in the taurine condition was observed. This finding was consistent across all three IHGs, despite the size of the vasodilatory response reducing across time in both conditions (Table 8.1). Previously, post-IHG exercise brachial artery hyperaemic responses have been partially attributed to production of metabolites that induce vasodilation, alongside the release of NO (Kagaya & Homma, 1997). As no changes in muscle metabolism were observed between conditions, this finding is suggestive of an endothelium-dependant mechanism, predominantly mediated by NO (Green et al., 2014; Sun et al., 2016) and an NO-mediated mechanism is also responsible for prolonged subcutaneous responses (Low et al., 2020). Given that NO and H_2S signalling are mutually inter-dependent (Nagpure & Bian, 2016; Szabo, 2017), the increase in resting SkBF and arterial reactivity could be explained by the same mechanisms (DiNicolantonio et al., 2017; Sun et al., 2016; Szabo, 2017; Zhao & Wang, 2002).

Based on the above evidence, taurine appears to augment vasodilation in skin micro-vessels, during rest and IHG exercise, among healthy individuals. In the resting state, this finding is favourable for maintenance of thermal balance. Since blood pressure was preserved, despite higher sweat losses in the taurine condition, these results also suggests that taurine's actions may have a homeostatic function, as previously speculated (Nishida & Satoh, 2009). In further support of this, greater SkBF vasodilation% of the forearm during the IHG exercise was observed following taurine supplementation in the current study. Increases in SkBF during IHG are observed at T_{sk} below 39°C (Kondo et al., 2003; McCord & Minson, 2005; Taylor et al., 1989) and are suggested to be due to withdrawal of adrenergic vasoconstrictor tone (McCord & Minson, 2005), to facilitate improved thermoregulatory defences during metabolic work. In the *in-vitro* animal model, taurine has been demonstrated to reduce adrenergic vasoconstriction (Denisov, 1998) and, therefore, may have the ability to facilitate its withdrawal, explaining the enhanced response during the IHG exercise. Similarly, SkBF had a faster return to baseline upon cessation of IHG in the taurine condition compared to placebo. This is indicative of a hastened recovery of the microvasculature to pre-exercising levels, e.g. return to homeostasis. This could relate to the reconstitution of vasoconstrictor tone and may, in part, be due to improved $Ca^{2+,}$ handing (Nishida & Satoh, 2009), and potential efficiency of reuptake at the sarcoplasmic reticulum within vascular smooth muscle (Dutka et al., 2014). A requirement of enhanced vasoconstriction and/or reduced vasodilation to achieve this, supports the notion that taurine's effects on vascular tone are not just vasodilatory (Nishida & Satoh, 2009; Simmonds et al., 2022), but appear to be condition specific. Whether in such

instances, taurine is affecting adrenergic vasoconstrictor tone, or the active vasodilatory system remains to be discovered.

Despite these novel and promising findings, there was no change in resting brachial artery diameter or blood flow between conditions, with the 2.4% (η_p^2 = 0.267) increase in brachial artery diameter being non-significant (p = 0.058). It might be expected that to achieve greater resting SkBF, as observed in the current study, vasodilation of major arteries would occur, alongside improved arterial blood flow (Johnson, 1996). Furthermore, taurine did not appear to influence any central cardiovascular parameters in the current study. Blood pressure and MAP were maintained in the taurine condition across all time points during the trial. With improved peripheral vasodilation, a corresponding reduction in central blood pressure might be expected (Munir et al., 2008). Indeed, mechanistically, this is often the explanation for how taurine exerts its anti-hypertensive effects (Sun et al., 2016; Waldron et al., 2018b). It has been linked to increased plasma H_2S, as described above, and angiotensin II antagonism (Dawson et al., 2000). In humans, the majority of literature regarding taurine's vasodilatory effects are in hypertensive individuals, or those suffering from other vascular comorbidities (Sun et al., 2016; Waldron et al., 2018b). Here, there is evidence that chronic oral taurine supplementation (1 to 12 weeks) lowers systolic blood pressure by approximately 3 mmHg (Waldron et al., 2018b). Potentially, its hypotensive effect is less pronounced during heat exposure, where preserving blood pressure is required to maintain sufficient SkBF perfusion and dissipate heat. Blood pressure maintenance is often reported during passive heating, despite large increases in cutaneous blood flow (Crandall & Wilson, 2015). Taurine does act to maintain blood pressure in certain conditions (Nishida & Satoh, 2009) and, theoretically, this would limit vasodilation of the major vessels (e.g. brachial artery), as demonstrated herein. It is also possible that the supine position of the participants, given the substantial effect this has on blood pressure maintenance via enhancement of venous return (Muntner et al., 2019), may have overridden any minor adjustments in blood pressure induced by taurine. It is, therefore, unlikely that taurine had any meaningful effect on blood pressure, despite the increase in peripheral vasodilation and fluid losses. Replication of these findings using continuous blood pressure measurement would more conclusively establish the temporal dynamics of blood pressure following taurine supplementation during heat exposure.

8.5.3. Cardiovascular responses

There were also no effects of taurine supplementation across parameters of cardiovascular function. As expected during passive heating, HR increased as the trial progressed (Crandall & Wilson, 2015), but to no larger degree in the taurine condition compared to placebo. This

was somewhat expected, considering non-significant HR findings from previous studies supplementing taurine during heat exposure (Page et al., 2019; Chapter Seven). Although significant, $\dot{Q}$ only marginally increased across the trial in both conditions, which was largely explained by the concomitant and relatively larger reduction in SV across time. At ambient temperatures up 46°C, only slight increases in $\dot{Q}$ would be expected (Damato et al., 1968), as observed here. The lack of change in HR and SV and, consequently, $\dot{Q}$ in the current study in response to taurine supplementation may be responsible for the null effect on brachial artery blood flow. Greater arterial blood flow is primarily facilitated by increased $\dot{Q}$ (Crandall & Wilson, 2015) and in the absence of such changes, it is unsurprising that this was not substantially enhanced. Greater SkBF may, therefore, simply be explained by a preferential redistribution of blood to the skin (the organ of immediate thermal gain) in the taurine condition (Rowell, 1974). Without further analysis of blood flow distribution throughout the body during passive heat stress in response to taurine supplementation, this is not definitive.

8.5.4. Metabolic responses

There was no difference between conditions in TSI%, [HHB] and [tHB] within the muscle of the forearm during the IHG exercise. This is indicative of a maintenance of skeletal muscle blood flow, despite increased distribution to the cutaneous vasculature following taurine supplementation, further highlighting its potential homeostatic role. Nevertheless, these results, along with no observable changes in pulmonary $\dot{V}O_2$ or RER between conditions, indicate that taurine did not alter muscle metabolism - including substrate utilisation - across the passive heating protocol or during the additional metabolic work stimulated by the IHG exercise. Previously, taurine has been shown to improve skeletal muscle contractility (Lim et al., 2018) and is also associated with improved oxidative metabolism (Hansen et al., 2010) and muscle function (Seidel et al., 2019). Indeed, in the exercising human, improvements in endurance performance (Waldron et al., 2018a) and changes in metabolic responses have been observed with taurine supplementation, such as increased fat (De Carvalho et al., 2018; Rutherford et al., 2010) and decreased carbohydrate oxidation (Simmonds et al., 2022) and oxygen consumption (Balshaw et al., 2013; Zhang et al., 2004). It appears that during exercise (i.e. increased metabolic work), taurine does have the potential to improve the efficiency of oxidative pathways (Hansen et al., 2006), though this was not observed in the current study. It should be stated, however, that only a subset of the data could be used in the analysis of muscle oxygenation variables, which limits the certainty of these conclusions. Elucidation of taurine's effects on metabolism in the heat may require an exercising state, where any

alterations in oxidative metabolism can be characterised using local and systemic measurements.

8.5.5. Core temperature responses

The current study suggests that taurine supplementation does not affect central cardiovascular function during passive heat exposure in healthy humans but does appear to affect peripheral vasodilation and sweat response. Ambient conditions permitting, this taurine-induced enhancement of SkBF and sweat rate would allow a greater capacity for dry and evaporative heat transfer. However, in very hot and/or humid environments there is a small temperature and/or vapour pressure gradient between the skin's surface and the ambient air. Consequently, the ability to dissipate heat and maintain thermal balance is reduced, despite the activation of compensatory heat loss mechanisms (vasodilation and sweating; Cramer & Jay, 2019). Therefore, the effectiveness of taurine for facilitating improved heat dissipation will be highly dependent on the specific ambient conditions. Indeed, in the current study, air temperature exceeded T_{sk} and, therefore, latent heat transfer (evaporation of sweat from the skin) was the only means by which to dissipate $\dot{H}_{prod}$. The LSR results suggest that taurine only augmented sweat rate on the lower half of the body (Figure 8.6), which was covered by reflective foil (low $\dot{E}_{max}$), limiting the evaporative cooling potential of the addition sweat production. Consequently, there was no demonstrable improvements in whole-body, resting thermal balance, represented by no changes in T_{core} between conditions, despite these enhanced heat loss mechanisms in response to taurine administration. This is contrary to the meta-analytical results regarding taurine in Chapters Four and Five, which is likely due to the differing experimental design. Nevertheless, the use of clothing or protective equipment that does not permit evaporative heat transfer, would not allow taurine-induced augmentation of sweating to provide a thermoregulatory benefit.

8.5.6. Practical implications

High ambient temperatures are becoming more common globally, due to climate change (Lee et al., 2023), which poses a substantial heat stress risk to a large proportion of the population (Epstein & Yanovich, 2019; Marx et al., 2021). Identifying a dietary strategy to help mitigate thermoregulatory strain in such conditions could be an easy and effective means by which to reduce this risk. Oral supplementation of taurine in hot environmental conditions offers a potential practical approach to offset the harmful effects of heat strain among healthy individuals. Nevertheless, further research is necessary to more fully elucidate its impacts on

thermoregulatory function, particularly in populations at greater risk of heat stress (e.g. older adults). Herein, several future research directions have been suggested, which will help to better understand its mechanisms of action during heat exposure.

8.5.7. Conclusion

Eights-days oral taurine supplementation (50 mg/kg) increased WBSL and leg LSR, suggesting both a systemic and local effect on sudomotor function in response to passive heat strain. Resting SkBF and CVC, as well as brachial artery and SkBF vasodilation% were also greater, indicating a vasodilatory enhancement of the peripheral vasculature during passive heat exposure and post-IHG exercise. Despite this, taurine did not affect resting brachial artery diameter and blood flow, blood pressure or other parameters of cardiovascular function (HR, SV and $\dot{Q}$). While taurine supplementation appears to confer some thermoregulatory benefits via augmentation of the sweating response and enhanced cutaneous vasodilation during thermal strain, there was no change in skin or T_{core}, suggesting these improvements had little overall influence on thermal balance, likely owing to the experimental design. These findings could have potential implications for individuals at risk of heat stress in environmental conditions that permit dry and evaporative heat dissipation. The mechanistic explanation for these observed effects and their efficacy in varying populations and real-world scenarios remains to be elucidated.

9. Chapter Nine – Discussion and future directions

9.1. General summary and discussion

This section will cover a general summary and discussion of the findings of the thesis and provide take-home messages. The work presented in this thesis comprehensively investigated the effects of the dietary supplement taurine on physiological and thermoregulatory responses during heat exposure.

9.1.1. Meta-analyses

Collectively, the two meta-analyses provide a comprehensive overview of the effects of various dietary supplements on endurance performance and thermoregulatory responses during heat exposure. Several supplements, such as caffeine, nitrate, and creatine, which have been demonstrated to be ergogenic in thermoneutral conditions, do not appear to provide a performance benefit in the heat. The limiting factors to exercise in the heat differ (Cheuvront et al., 2010; González-Alonso et al., 2008; Nybo, 2010; Nybo et al., 2011; Thompson, 2006; Tucker et al., 2004) and, consequently, the mechanisms of action of certain supplements are either more or less effective in these conditions. Indeed, amino acids (BCAAs, tyrosine and taurine) appeared to induce the greatest performance benefits in hot conditions, yet are not among the dietary supplements commonly selected by athletes (Knapik et al., 2016; Wardenaar et al., 2017), likely due to their equivocal empirical evidence in thermoneutral conditions. Nevertheless, the performance effects of these supplements were non-significant and, therefore, further research is required to confirm these findings.

Caffeine supplementation increased T_{core} responses during both rest and exercise, which has potential health and performance consequences. Available heat storage capacity is depleted as T_{core} rises, which leads to an earlier onset of hyperthermic symptoms and consequently, deleterious effect on exercise performance (González-Alonso et al., 1999). Based on the findings herein, caffeine should be avoided in scenarios where there is a significant risk of heat stress. The potential thermogenic effects of caffeine when ingested in hot conditions is important to highlight to athletes, military personnel, occupational workers and the general population, so as not to exacerbate symptoms of heat strain. Given that heat illness during endurance events in hot environments is common and presents a risk to sports (Howe & Boden, 2007) or tactical personnel (Armed, 2017; Bricknell, 1996; Cox et al., 2016), such outcomes should be more clearly recognised in dietary guidance.

Dietary nitrate and other supplements taken for their effects on NO bioavailability, did not improve thermoregulation in the heat (Chapter Five), likely explaining the null performance effects for nitrate (Chapter Four). No significant augmentation of the sweating response was observed and no reduction in T_{core}, suggesting enhanced heat dissipation was not induced through dry or evaporative pathways. This was surprising given the vital role NO plays in peripheral vasodilation, as well as its potential effects on sweat gland function (Fujii et al., 2014; Stapleton et al., 2014a). These findings, in combination, therefore question whether nitrate supplementation has the capacity to aid thermal balance in hot environmental conditions.

A number of supplements with anti-oxidative and anti-inflammatory properties provided beneficial effects on end T_{core}, but no significant increases in sweating responses were observed for any of these supplements. The potential thermolytic effects are likely through improved redox balance and attenuation of endogenous pyrogenic cytokines (Gulcin, 2020; Vybíral et al., 2005). Further, not all anti-oxidative and anti-inflammatory supplements improved thermal balance, with results displaying large heterogeneity. As such there are currently equivocal and largely non-significant findings for the majority of these supplements, which highlights the need for substantiation of results and additional research within this area. Similarly, several supplements taken for their hyperhydrating effects improved thermal balance, as might be expected due to an improved heat carrying capacity of the body and/or blood (Kay & Marino, 2000; Kilduff et al., 2004; Sawka, 1992). Further, plasma volume expansion may improve fluid availability to active sweat glands (Fortney et al., 1981; Wong & Hollowed, 2017), thus increasing sweat production, which is reflected in the WBSR findings for combined glycerol and creatine.

Some amino acids, such as GABA, whey protein and taurine lowered T_{core} responses, indicating an influence on thermal balance. The majority of amino acids appear to exert their effects via centrally-mediated mechanisms for both performance (e.g. tyrosine and BCAAs through attenuation of central fatigue) and thermoregulation (e.g. GABA via actions within the hypothalamus). Despite their potential ergogenic effects in the heat tyrosine and BCAAs did not induce any thermoregulatory benefits. Indeed, none of these supplements affected WBSR or LSR, indicating no benefits were induced through this thermoeffector response. Nevertheless, taurine supplementation resulted in an increased sweating rate and an earlier onset of sweating, likely facilitating improved evaporative heat transfer. This may explain the reduced T_{core}, as a greater thermoregulatory capacity would delay any rise, although these findings were from a single study.

These meta-analyses provide insight into the effects of supplements in the heat that has not been previously established. Overall, there is insufficient research regarding the effects of dietary supplements in hot conditions. Both their performance and thermoregulatory effects need to be more thoroughly established. This is important for performance success, but also for reducing the risk of heat illness and subsequent negative health consequences. Finally, the difference in dietary supplement efficacy in varying environmental conditions should be reflected in current dietary supplements guidelines for sports and tactical personnel.

Take-home messages

- Exercising in the heat significantly influences the ergogenic efficacy of many dietary supplements, suggesting that performance findings from research conducted on certain supplements in thermoneutral conditions are not necessarily transferrable to other environmental conditions.
- Certain supplements, such as caffeine, nitrate and creatine, lack sufficient data to support their use as ergogenic aids in the heat, despite their efficacy in thermoneutral conditions.
- Some amino acids might offer the greatest performance benefits in the heat.
- Caffeine significantly and consistently increased T_{core} responses across both meta-analyses, demonstrating a thermogenic effect when ingested in the heat. Consequently, intake should be avoided during periods of heat exposure, so as not to exacerbate symptoms of heat stress.
- Select amino acids (e.g. taurine and GABA), anti-oxidants and anti-inflammatories (e.g. oligonol and catechin), and supplements affecting fluid balance (e.g. sodium citrate and combined glycerol and creatine) may offer the greatest thermoregulatory benefits during heat exposure. Conversely, supplements enhancing NO bioavailability (e.g. nitrate) appear to have no effect on thermal balance.
- A potential risk is posed to the general population and those in physical performance domains (i.e. athletes or military personnel) due to the limited guidance on how different supplements may affect thermoregulatory functioning in hot environments.

9.1.2. Primary research studies

Metabolic heat production and $\dot{E}_{req}$ have previously been established as the primary drivers of the thermal sweating response (Cramer & Jay, 2014, 2016; Gagnon et al., 2013). Therefore, in many experimental trials aimed at evaluating the sweating response, exercise is controlled using $\dot{H}_{prod}$. Work within this thesis has shown for the first time that absolute and relative $\dot{H}_{prod}$

and $\dot{E}_{req}$ demonstrate sufficient inter-day reliability to control the thermal sweating response, which is highly important for such studies. Further, it was established that the sweating measures of WBSL, LSR and SGA have inter-day reliability sufficient to detect changes in thermal sweating that might be expected during exercising heat stress. This is vital for their use in experimental studies, where trials will often span more than one day and be up to weeks apart. These methods can now be used to assess thermal sweating with the knowledge they will produce sufficiently reliable results. However, all measures of reliability at rest were considered insufficient to detect changes in thermal sweating, indicating that they should not be used to characterise the sweating response in those conditions. Knowledge that certain measures of, and the primary drivers of the sweating response ($\dot{H}_{prod}$ and $\dot{E}_{req}$), display sufficient inter-day reliability, enabled accurate characterisation of taurine's effects on the sweating response in the first experimental study of the thesis (Chapter Seven). Conversely, LSR using the absorbent patch technique and SGA were not assessed in the passive heating study (Chapter Eight), due to these findings. Additionally, having evaluated the reliability of varying iodine-paper sizes it was established that identifying the optimal 3 x 3 cm or 1 x 1 cm area of sweat gland density (i.e. the densest area) produced the most consistent results. This is likely due to the pulsatile and variable nature of sweat gland innervation (Subramanian et al., 2020). Therefore, in the first experimental study, 9 x 9 cm iodine-paper sizes were used, and the optimal 3 x 3 cm and 1 x 1 cm area of sweat gland density identified within it to establish the effect of taurine supplementation on SGA.

Not all aspects of the sweating response have previously been associated with $\dot{H}_{prod}$ and $\dot{E}_{req}$. Through establishing WBSL, LSR and SGA at varying exercise intensities (i.e. varying levels of $\dot{H}_{prod}$), the relationship between the two can be deduced. The findings that WBSL and LSR (as measured with the absorbent patch technique) are positively related with these drivers supports findings from previous literature. Additionally, for the first time, this work has established that SGA is associated with $\dot{H}_{prod}$ and $\dot{E}_{req}$. The relationship between $\dot{H}_{prod}$ and $\dot{E}_{req}$ and multiple aspects of the sweating response has now been comprehensively evaluated.

Take-home messages

- Absolute and relative $\dot{H}_{prod}$ and $\dot{E}_{req}$ demonstrate sufficient inter-day reliability to control the thermal sweating response, and sweating measures of WBSL, LSR and SGA have inter-day reliability sufficient to detect changes in thermal sweating that might be expected during exercising heat stress.
- Establishing the optimal 3 x 3 cm and 1 x 1 cm area of sweat gland density within a large paper size (e.g. 6 x 6 cm or 9 x 9 cm) is the most reliable means of assessing SGA.

- Control of $\dot{H}_{prod}$ and $\dot{E}_{req}$ allows for sufficient control of WBSL, LSR (as measured with the absorbent patch technique) and SGA (as measured by the modified iodine-paper technique), permitting evaluation of multiple changes in these aspects of the sweating response through interventions (e.g. dietary supplementation).

One of the most significant findings of the work within this thesis is that oral taurine supplementation augments multiple aspects of the thermal sweating response. In both Chapter Seven and Eight, a greater systemic sweating response through the measure of WBSL was observed. As exercise was controlled using $\dot{H}_{prod}$ (~200 W/m_2) in the first experimental study, and participants were at rest in the second, the sweating response was elevated while the drivers of sweating were stable, indicating an augmentation of the sweating response not affected by this influencing factor. In Chapter Seven, LSR and SGA were also greater in the taurine condition, with these changes larger in magnitude than CVs established for these measures in Chapter Six, suggesting a genuine change in thermal sweating (Figure 9.1.a). A greater sweating response should allow for improved evaporative heat loss and, consequently, improved thermal balance. Indeed, enhanced evaporative heat dissipation at the skin and subsequent decreased heat storage, as modelled by partitional calorimetry, was identified in Chapter Seven. This translated to the breakpoint of compensability occurring at a higher ambient vapour pressure, indicating a heightened ability to tolerate thermally stressful environmental conditions.

In Chapter Eight using the ventilated capsule technique, the leg was the only site shown to have a taurine-induced increase in LSR. This was also the only measured site which likely had a greater $\dot{E}_{req}$. Further, sweat rate in the taurine condition increased relative to placebo as the trial and heat strain progressed. Additionally, WBSL was greater in the first experimental study during exercising, rather than during passive heat stress (26.6 *vs* 16.2% increase). Collectively, this provides evidence for the assertion that taurine may be heat strain- or $\dot{E}_{req}$-mediated, which is a novel finding and one that requires further investigation. If substantiated it suggests that taurine supplementation may be most effective in exercising or environmental conditions that elicit heightened thermal strain, with a greater requirement for evaporative cooling to offset $\dot{H}_{prod}$. These findings also pose questions regarding taurine's mechanisms of action and are suggestive of a predominantly peripheral effect, despite earlier work indicating the possibility of a centrally-mediated mechanism (Page et al., 2019; Chapter Seven). Both central and peripheral effects remain plausible and it is reasonable to propose that increased thermal sweating occurs owing to a combination of several factors, which is consistent with the numerous biological roles ascribed to taurine (Huxtable, 1992). Investigating these in

greater depth was beyond the scope of this thesis but, nevertheless, multiple possible mechanisms of action have been proposed and several examined. While it does not currently appear that plasma volume expansion or increased SkBF are responsible for the increased sweating response following taurine supplementation, these cannot be entirely ruled out without more specific investigation.

Take-home messages

- Taurine supplementation has been demonstrated to augment thermal sweating during heat exposure, across multiple experimental studies. This enhancement is beyond the measurement error of the techniques used to assess this response, indicating a genuine increase.
- Several mechanisms of action have been proposed to explain this response; however, additional research is required to elucidate this and provide further mechanistic understanding.
- Taurine supplementation may, therefore, provide a thermoregulatory benefit in hot conditions that permit evaporative heat transfer, which has potential implications for athletes, military personnel and occupational workers expected to perform while exposed to such environmental conditions. Nevertheless, its applicability in such scenarios requires further examination.
- Inflection protocols designed to establish the breakpoint of compensability can be utilised to test the acute effect of a supplement or other intervention on thermal tolerance and partitional calorimetry can appropriately model heat transfer alongside this.

Another major novel finding of the thesis is that taurine increased SkBF and CVC during heat exposure. This is believed to be the first study to establish an *in-vivo* effect of taurine on cutaneous vasodilation, especially in the context of thermoregulatory responses, and we have advanced the field in this area. These results develop upon previous research, in which *in-vitro* studies have repeatedly demonstrated taurine's ability to induce vaso-relaxation in rodent macro-vessels (Yildiz & Ulusoy, 2022). This experimental study expands upon the work of Sun et al. (2016) who observed a greater FMD response following 12 weeks taurine supplementation. Here, eight days of taurine supplementation was demonstrated to elicit a similar reactive vasodilatory response in the brachial artery following partial arterial occlusion from an IHG. Collectively, these findings substantiate taurine's vaso-active role and suggests intake has a favourable effect on this thermoeffector response during heat exposure (Figure 9.1.b).

A surprising finding was that T_{core} was not significantly reduced in either experimental study following taurine supplementation (Figure 9.1.d), despite an improved thermoeffector response observed in both. In Chapter Seven, there was a trend for a lower T_{core} response, which suggests the greater sweat production and evaporative heat transfer elicited small but insignificant effects on T_{core}. Further, the lack of change in T_{core} in Chapter Eight could be attributed to the experimental design of the study, as environmental conditions did not allow for dry heat dissipation, and sweating appeared to be augmented only on the lower half of the body, under the foil covering, where evaporation could not take place. Therefore, despite these findings, taurine's thermolytic effects cannot be ruled out and further investigation into this must be undertaken in studies designed to permit dry and evaporative heat transfer.

Despite *in-vitro* studies repeatedly demonstrating taurine's role in cardiac contractile function (Schaffer et al., 2010; Satoh & Sperelakis, 1998), and some *in-vivo* evidence to suggest taurine has an inotropic effect (Satoh & Sperelakis, 1998), no such observations were found in the current thesis (Figure 9.1.e). Heart rate, SV and $\dot{Q}$ were unchanged across the passive heating protocol (Chapter Eight), and HR was also no different during exercising heat stress (Chapter Seven). Another unanticipated finding was that taurine did not lower blood pressure during passive heating, as this is one of taurine more established effects when supplemented in thermoneutral conditions (Waldron et al., 2018a; Figure 9.1.c). Any small hypotensive effects taurine may have induced, could potentially have been overridden by the supine position the participants were in. It is also possible that the hypotensive effect of taurine is less pronounced during heat exposure, where preserving blood pressure is required to maintain sufficient SkBF perfusion for heat dissipation. Indeed, blood pressure maintenance is often reported during passive heating, despite large increases in cutaneous blood flow (Crandall & Wilson, 2015). Taurine has been reported to act to maintain blood pressure in certain conditions (Nishida & Satoh, 2009) and, theoretically, this would limit vasodilation of the major vessels (e.g. brachial artery), as demonstrated herein. Further research utilising continuous blood pressure monitoring in differing body positions during heat exposure would provide additional mechanistic insight into these findings.

This thesis revealed that during heat exposure, taurine does not appear to affect whole-body metabolism, as there were no significant changes in $\dot{V}O_2$ and RER (Chapter Seven and Eight) or $\dot{V}O_{2max}$ and the $\dot{V}O_2$-WR relationship in the incremental tests (Chapter Seven; Figure 9.1.f). Further, there was no difference in muscle oxygenation between conditions during the IHG in Chapter Eight, indicating no change in muscle metabolism during exercise. Nevertheless, previous observations of improved endurance performance (Waldron et al., 2018a) and greater maximal oxygen consumption (Balshaw et al., 2013; Zhang et al., 2004), alongside findings of alterations in metabolic responses, such as substrate utilisation (De Carvalho et

al., 2018; Rutherford et al., 2010; Simmonds et al., 2022) challenge this conclusion. It appears that during exercise (i.e. increased metabolic work), taurine does have the potential to improve the efficiency of oxidative pathways (Hansen et al., 2006); therefore, additional research is required to understand why this does not occur in the heat.

Take-home messages

- Taurine supplementation increased peripheral vasodilation and brachial artery reactivity following partial occlusion, demonstrating it has an *in-vivo* vaso-active function in the heat, although corroboration of these findings and further mechanistic insight is necessary.
- Taurine supplementation may, improve dry heat transfer at the periphery through enhanced cutaneous vasodilation, thus contributing to the maintenance of thermal balance in hot environmental conditions. However, this requires further investigation through modelling via partitional calorimetry and in ambient temperatures which permit dry heat dissipation.
- Taurine did not elicit a hypotensive effect or changes in HR, SV and $\dot{Q}$ at rest, or changes in HR, $\dot{V}O_{2max}$, the $\dot{V}O_2$-WR relationship and muscle oxygenation during exercise in the heat. This suggests these responses remained stable, despite other meaningful changes, such as increased fluid loss (i.e. sweating) and peripheral vasodilation.
- Taurine appears to produce these thermoeffector responses (i.e. sweating and peripheral vasodilation) without inducing other adverse effects or alterations in cardiovascular or metabolic variables, indicating the safety of its ingestion in hot environmental conditions.
- These findings are limited to a laboratory setting and are based on the assumptions that the skin is exposed to the ambient environment to facilitate heat transfer and that the individuals are euhydrated, for example. The efficacy of taurine at improving thermoregulation in real-world scenarios, where individuals may be wearing impermeable clothing or have a variable hydration status, is unknown.

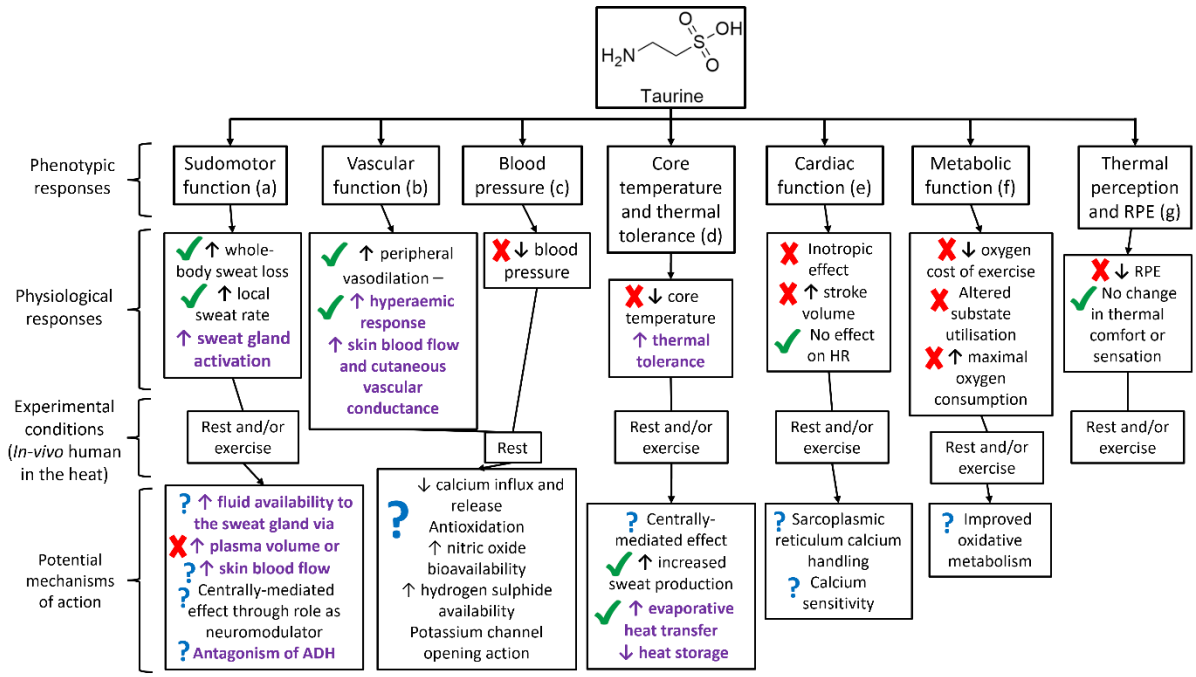


Figure 9.1. The effect of oral taurine supplementation on thermoregulatory phenotypic responses during heat exposure and comparisons to previous literature.

Green ticks and red crosses denote findings in the thesis that are in agreement or disagreement with previous literature, respectively. Blue question marks denote uncertainty regarding a finding. Purple text states knowledge that has been added to the literature through the experimental studies conducted within this thesis.

9.1.3. Summary

The meta-analyses within this thesis revealed that the performance enhancing effects of many dietary supplements are affected by the heat. Several supplements demonstrated increased ergogenicity (several amino acids), whereas the efficacy of others was reduced compared to thermoneutral conditions (e.g. caffeine, creatine and nitrate). Select animo acids, anti-oxidants and anti-inflammatories, and supplements affecting fluid balance provided a thermoregulatory benefit during heat exposure, whereas supplements increasing NO bioavailability did not. Caffeine had a thermogenic effect when ingested in the heat and, therefore, should be avoided in hot conditions, so as not to exacerbate heat stress. The experimental findings of this thesis suggest taurine has the ability to influence both thermoeffector responses of sweating and vasodilation during heat exposure. Taurine is potentially most effective at improving thermal sweating during periods of heightened thermal stress, though this requires additional investigation. Taurine appears to improve thermal tolerance by enhancing evaporative cooling and reducing heat storage. Further understanding regarding the mechanisms of action

responsible for the enhancement of thermal sweating and peripheral vasodilation is required. However, based on the findings of the current thesis, taurine supplementation may offer a dietary supplementation strategy to support thermoregulation for athletes, occupational workers and military personnel who endeavour to perform in hot environmental conditions, but further translational work is required to understand its place in these scenarios. It should be noted taurine would only provide a thermoregulatory benefit in conditions that permit dry and evaporative heat dissipation.

9.2 Future work

9.2.1 Dietary supplements in the heat

Chapters Four and Five investigated the effects of various dietary supplements on endurance exercise performance, T_{core} and sweating responses during heat exposure. Whilst the current thesis used these meta-analytical findings to investigate taurine supplementation in the heat, it was apparent that a number of dietary supplements' ergogenic effects in the heat, such as caffeine, and select amino acids, require further investigation. This includes investigation of their mechanistic action, as many of the included studies require replication to form more definitive conclusions. Several supplements, displaying the greatest thermoregulatory responses during heat exposure, should be investigated further. Indeed, future research should focus on the thermolytic effects of various supplements, such as GABA, oligonol and catechin in varying conditions, alongside further mechanistic insight into these responses. Additionally, the meta-analyses revealed that the effects of chronic supplementation amongst the majority of dietary supplements when taken in the heat is almost completely unknown. Evaluation of this is of particular importance for the most thermally efficacious supplements, such as taurine (as detailed in this thesis), and other amino acids (e.g. BCAAs and tyrosine for endurance exercise performance) and GABA for its thermolytic effects. The mechanistic reasoning for caffeine's thermogenic effects is necessary, owing to its widespread supplementation amongst athletes and the general population.

The meta-analyses revealed that future research should attempt to understand the efficacy of selected supplements (e.g. caffeine, BCAA, GABA, taurine) during a wider range of exercising conditions, encompassing a variety of physiological demands, particularly prolonged exercise where these effects are largely unknown. It was also clear that ecologically valid conditions were rarely considered in this form of research and there is an ongoing need to establish their 'real-world' effectiveness. This may facilitate the translation to the end user and enable the provision of practical advice and application to athletes, workers and the general population.

This is important, since a vast majority of the global population (80 to 85%; Heckman et al., 2010; Mitchell et al., 2014) regularly take dietary supplements and their intake in also common amongst athletes. The climate is warming, and this has implications for the working conditions of many occupations, such as military personnel, as well as other settings, such as athletic competitions, which are taking place in hot environmental conditions, exposing athletes to enhanced thermal strain. This necessitates more thorough understanding of the effects various supplements may have on thermoregulation and thermal balance during passive heat exposure and exercising heat stress.

9.2.2. The effect of taurine on thermal sweating and potential mechanisms of action

Chapters Seven and Eight investigated the effects of taurine supplementation on thermoregulatory responses during heat exposure. These studies established that taurine induces thermal sweating and cutaneous vasodilation, but to further characterise these responses additional research is required. As previously stated, taurine appears to be more efficacious in more thermally stressful scenarios and, therefore, future studies investigating the magnitude of augmented sweating in response to taurine supplementation at varying degrees of heat strain would help elucidate this response. Its effects on thermal sweating and subsequent heat balance in more ecologically valid conditions is also unknown, and is ultimately of utmost importance. Further, the underpinning mechanisms of action responsible for the taurine-induced augmented thermal sweating responses are largely speculative. Future studies should endeavour to establish: 1) whether taurine supplementation influences plasma volume acutely; 2) whether taurine-induced augmentation of SkBF directly improves sudomotor function; 3) whether taurine augments thermoeffector responses via a central mechanism.

9.2.3. The effect of taurine on vascular function during heat stress

Chapter Eight investigated the effects of taurine supplementation on vascular function, at the micro- and macro-level, during passive heating. The findings that taurine elicits cutaneous vasodilation requires replication before any definitive conclusions can be formed, but is the first evidence that an oral supplement directly influences this key heat loss avenue. Further, it is important to characterise the effects of taurine on vasodilation during exercise in the heat, alongside heat transfer modelling, which can be reliably performed via partitional calorimetry (Chapter Six). It is yet to be established whether taurine can meaningfully improve dry heat transfer through this thermoeffector response, and whether this same response is observed

during exercise. Further, the lack of a change in resting blood pressure following taurine supplementation in the heat was unexpected and requires further insight through continuous blood pressure monitoring in a similar scenario. Similar to taurine's effects on sudomotor function, the mechanisms underpinning *in-vivo* vasodilation are unknown but could relate to H_2S availability. Therefore, greater insight into these mechanisms is necessary to more thoroughly understand this response.

Appendices

A – Letters confirming ethical approval for all experimental studies within the thesis

Chapter Six ethical approval

LEAD APPLICANT NAME: Jennifer Peel
DISCIPLINE/DEPARTMENT: SPEX
PROJECT TITLE: Inter-day reliability of sweat gland recruitment and sweat content measurement techniques during rest and exercise
APPLICATION REFFERENCE NUMBER: Jennifer_Peel_25-11-20

Swansea University
Prifysgol Abertawe

Date of review board: December
Committee members in attendance: Chairs

Date: 16th December 2020

Dear Jennifer,

Thank you for your recent ethics application.

This decision letter is to inform you that the ethics application for the above titled project has been reviewed and approved. The ethical approval number for this application is JP_25-11-20 approved from 16-12-20 – end of approval 01-12-22.

This letter is for Swansea University, College of Engineering Research Ethics and Governance approval only. Local Health and Safety, in addition to appropriate risk assessment guidelines are required separate to this approval, unless otherwise stated herein, and must be adhered to.

Associated researchers must not deviate from the approved protocol or extend beyond the approval end date. Any desired deviations or approval date extensions are subject to the ethical approval amendment process. Upon completion of the approved project researchers responsible for this application must submit a final (short) statement to the ethical committee stating the completion of the project, unless a time extension is being requested through the amendment process.

Any significant un-anticipated adverse effects/events (i.e. not those predicted and stated in section 8 of the ethics application form) must be reported to the Ethics committee upon researcher realisation (email: coe-researchethics@swansea.ac.uk; with the subject title including the study approval number followed by "Adverse Effects/Events").

If you have any further questions relating to your application, please contact: coe-researchethics@swansea.ac.uk.

Please keep note of your approval number for future reference and correspondence relating to this application.

Best of luck with your research.

Warm regards,

Aynsley Fagan

(on behalf of the College of Engineering Research Ethics and Governance Chair)

College of Engineering Ethics and Governance Committee Administrator
College of Engineering | Y Coleg Peirianneg
Swansea University | Prifysgol Abertawe
Fabian Way | Ffordd Fabian Crymlyn Burrows
Swansea | Abertawe
Wales | Cymru
SA1 8EN

Email: coe-researchethics@swansea.ac.uk.

LEAD APPLICANT NAME: Jennifer Peel
DISCIPLINE/DEPARTMENT: SPEX
PROJECT TITLE: The effect of chronic oral taurine and acute caffeine supplementation on thermoregulatory responses and exercise tolerance in the heat
APPLICATION REFFERENCE NUMBER: Jennifer_Peel_30-10-20b

Swansea University
Prifysgol Abertawe

Date of review board: February
Committee members in attendance: Chairs

Date: Friday 18th February

Dear Jennifer,

Thank you for your recent ethics application.

This decision letter is to inform you that the ethics application for the above titled project has been reviewed and approved. The ethical approval number for this application is JP_30-10-20b approved from 18/02/22 – end of approval 01/02/24. Please see reviewer document for more information.

This letter is for Swansea University, College of Engineering Research Ethics and Governance approval only. Local Health and Safety, in addition to appropriate risk assessment guidelines are required separate to this approval, unless otherwise stated herein, and must be adhered to.

Associated researchers must not deviate from the approved protocol or extend beyond the approval end date. Any desired deviations or approval date extensions are subject to the ethical approval amendment process. Upon completion of the approved project researchers responsible for this application must submit a final (short) statement to the ethical committee stating the completion of the project, unless a time extension is being requested through the amendment process.

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If you have any further questions relating to your application, please contact: coe-researchethics@swansea.ac.uk.

Please keep note of your approval number for future reference and correspondence relating to this application.

Best of luck with your research.

Warm regards,

Aynsley Fagan

(on behalf of the College of Engineering Research Ethics and Governance Chair)

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College of Engineering | Y Coleg Peirianneg
Swansea University | Prifysgol Abertawe
Fabian Way | Ffordd Fabian Crymlyn Burrows
Swansea | Abertawe
Wales | Cymru
SA1 8EN

Email: coe-researchethics@swansea.ac.uk.

Chapter Eight ethical approval

LEAD APPLICANT NAME: Jennifer Peel
DISCIPLINE/DEPARTMENT: SPEX
PROJECT TITLE: The effect of chronic oral taurine supplementation on thermoregulation during passive heat stress
APPLICATION REFFERENCE NUMBER: JP_31-01-23b

Swansea University
Prifysgol Abertawe

Date of review board: March
Committee members in attendance: Rachel Churm

Date: Wednesday 29th March 2023

Dear Jennifer,

Thank you for your recent ethics application.

This decision letter is to inform you that the ethics application for the above titled project has been reviewed and approved. The ethical approval number for this application is JP_31-01-23b approved from 29/03/23 end of approval 01/02/23.

This letter is for Swansea University, College of Engineering Research Ethics and Governance approval only. Local Health and Safety, in addition to appropriate risk assessment guidelines are required separate to this approval, unless otherwise stated herein, and must be adhered to.

Associated researchers must not deviate from the approved protocol or extend beyond the approval end date. Any desired deviations or approval date extensions are subject to the ethical approval amendment process. Upon completion of the approved project researchers responsible for this application must submit a final (short) statement to the ethical committee stating the completion of the project, unless a time extension is being requested through the amendment process.

Any significant un-anticipated adverse effects/events (i.e. not those predicted and stated in section 8 of the ethics application form) must be reported to the Ethics committee upon researcher realisation (email: coe-researchethics@swansea.ac.uk; with the subject title including the study approval number followed by "Adverse Effects/Events").

If you have any further questions relating to your application, please contact: coe-researchethics@swansea.ac.uk.

Please keep note of your approval number for future reference and correspondence relating to this application.

Best of luck with your research.

Warm regards,

Aynsley Fagan

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Fabian Way | Ffordd Fabian Crymlyn Burrows
Swansea | Abertawe
Wales | Cymru
SA1 8EN

Email: coe-researchethics@swansea.ac.uk.

B – Analysis of sweat gland activation using ImageJ

- Within the software ImageJ the image was imported (File toolbar – Open – desired image selected).
- The image type was set to 8-bit grayscale (Image toolbar – Type – 8-bit) and converted to binary (Process toolbar – Binary – Make binary). The dots indicating activate sweat glands were now displayed in black on a white background.
- The rectangular box on the left-hand side below the toolbar was selected, which allowed for the desired squared area on the image to be selected for analysis (i.e. 9 cm^2).
- The number of active sweat glands were counted (Analyze toolbar – Analyze Particles). The lower and upper size limit for the pixel area was set at 10-1000. 'Display results', 'clear results', 'exclude on edges' and 'record starts' were selected, alongside 'Outlines' under the 'Show' menu. Once these setting were established 'Ok' was selected and a display box appeared presenting the number of dots counted within the desired area of the image.
- The surface area covered was established (Image toolbar – Adjust – Threshold). A displayed box appeared presenting the percentage of the area covered in dots within the desired area of the image.
- These steps were repeated for analysis of each squared area and each image.

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