Thermoregulation in male endurance runners: Role of skin temperature during an incremental maximal exercise test

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TESIS DOCTORAL

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THEREMOREGULATION IN MALE ENDURANCE RUNNERS: ROLE OF SKIN TEMPERATURE DURING AN INCREMENTAL MAXIMAL EXERCISE TEST

INTERNATIONAL PhD

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"La falta de actividad destruye la buena condición de cualquier ser humano, mientras que el movimiento y el ejercicio físico metódico la guardan y la preservan"

Platón (427 A.C.- 347 A.C. Filósofo Griego)

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GLOSSARY OF ABBREVIATIONS AND ACRONYMS

Symbol	Description
ACSM	American College of Sport Medicine
ADP	adenosine diphosphate
AMP	adenosine monophosphate
ATP	adenosine triphosphate
BM	body mass
BMI	body mass index
BF %	body fat percentage
BS	body surface
BT	body temperature
BP	blood pressure
C	convection
CHO	carbohydrate
CHOox	carbohydrate oxidation
CHF	congestive heart failure
cm	centimeter
CO	crossover
CO_2	carbon dioxide production
CRF	cardiorespiratory fitness
Cres	convective heat exchange
CSA	cross-sectional area
CVD	cardiovascular disease
CV	coefficient of variation
CVR	cardiovascular risk factors
DEE	daily energy expenditure
DMH	dorsomedial nucleus of the hypothalamus
EE	energy expenditure
E_{res}	evaporative heat loss from the respiratory tract
E_{sk}	evaporative heat loss from the skin
ET	endurance training
FATox	fat oxidation rate

FAT_{max}	maximal fat oxidation rate
FAT_{min}	minimal fat oxidation rate
FFAs	free fatty acids
g	gram
GABA	gamma-aminobutyric acid
GPR81	G-protein- coupled receptor
H+	hydrogen ion
HF	high fit
HR	heart rate
HR_{max}	maximum heart rate
HR_{peak}	peak heart rate
HRR	heart rate reserve
HR_{max} %	percent of maximal heart rate
HT	high trained
IMP	inosine phosphate
IMTG	triglycerides
ISAK	International society for the advancement of Kinanthropometry
K	conduction
Kg	kilogram
$Kg \cdot m^{-2}$	meters squared
$[La^{-}]$	blood lactate concentration
$[La^{-}]_{max}$	maximal blood lactate concentration
LT	lactate threshold
m	meters
M	metabolic rate/ metabolic energy expenditure
MET	metabolic equivalent of task
MF	moderately fit
Min	minute
Mins	minutes
ml	milliliter
MA	moderately active
MF	moderately fit
MFO	maximal fat oxidation

MVV	maximal voluntary ventilation
NPHA	anterior hypothalamus preoptic nucleus
NST	non-shivering thermogenesis
PA	physical activity
PCr	phosphocreatine
PE	physical exercise
Pi	inorganic phosphate
PO	power output
PS	professional soccer
Q	cardiac output
r	Pearson correlation coefficient
R	radiation
RER	respiratory exchange ratio
Rd	rate of disappearance
RPE	rate of perceived exertion
S	rate of body heat storage
SD	standard deviation
SEM	standard error
SkBF	skin blood flow
$Speed_{peak}$	peak speed
SV	stroke volume
Ta	environment temperature
TR	thermoregulation
Tc	core temperature
TRP	transient receptor potential
Ts	shell temperature
Tsk	skin temperature
$Tsk_{baseline}$	baseline skin temperature
Tsk_{final}	final skin temperature
Tskm	mean skin temperature
Tsk_{peak}	peak skin temperature
T2DM	type 2 diabetes mellitus
VE	ventilation

VO2	oxygen uptake
VO_{2max}	maximal oxygen consumption/aerobic fitness/fitness level
VO_{2peak}	peak oxygen consumption/aerobic capacity/fitness level
VO_{2max} %	percent of maximal oxygen uptake
VO_{2R}	maximal oxygen uptake reserve
VT	ventilatory threshold
W	watts
Wk	external work
W/kg	kilogram of total body mass
^{13}C	carbon isotope

1. ABSTRACTS

1.1 English version

During endurance exercise, Tsk plays a fundamental role in body temperature regulation. Environmental temperature is the biggest determinant of Tsk, which is the result of the balance between metabolic heat production and heat dissipation to the environment. During exercise, Tsk response might be influenced by oxygen consumption (VO_{2peak}) and the metabolic flexibility, both powerful predictors of aerobic performance. Increased skin thermoregulatory capacity and FATox rates are characteristics of highly trained athletes, while decreased oxidative capacity and Tsk rates and higher [La-] concentrations are characteristics of individuals with low aerobic capacity, at the same absolute submaximal exercise intensities. The purpose of this project was to analyze and compare the dynamic of Tsk in different populations with different metabolic responses during an incremental maximal stress treadmill test. For this purpose, we performed two studies. The first study analyzed and compared the correlation between Tsk and cardiorespiratory variables in high fit (HF) (n= 35; VO_{2peak} = 56.62 ± 4.31 ml/kg/min) and moderately fit MF (n = 44; VO_{2peak} = 47.86 ± 5.29 ml/kg/min) male endurance runners during an incremental test with stages of 2 mins until exhaustion, followed by a recovery period of five minutes. The second study analyzed and compared the correlation between Tsk and metabolic flexibility by measuring [La^-] concentrations along with FATox and CHOox rates in high trained (HT) (n= 22; VO_{2peak} = 58.57 ± 2.33 ml/kg/min) competitive endurance runners, moderately active (MA) (n= 20; VO_{2peak} = 49.07 ± 4.67 ml/kg/min) runners and professional soccer (PS) (n= 23; VO_{2peak} = 53.34 ± 3.67 ml/kg/min) players during an incremental maximal test with stages of 3 mins until exhaustion, followed by a recovery period of five minutes. Results of the first study revealed that the MF group exhibited lower $VO_{2peak,}$ $speed_{peak}$, Ventilation (VE) and higher body mass index (BMI) and fat mass % than the HF group (all $p < 0.001$). Tsk was significantly higher at baseline, and at 60% and 70% of peak workload (all $p < 0.05$). Results of the second study revealed that MA group exhibited lower $VO_{2peak,}$ $speed_{peak}$, and higher BMI and fat mass % (all $p < 0.05$) than both HT and SP groups. There were correlations between Tsk with FATox and CHOox rates and [La-] concentrations for all data points of all groups (all $p < 0.001$). These findings indicate that higher VO_{2peak} and FATox rates and lower [La-]

concentrations were associated with increased Tsk during incremental maximal exercise across individuals of widely different metabolic capabilities. These differences should be taken into account in the training and nutritional strategies for enhancing endurance and team sports.

1.2 Versión en castellano

Durante el ejercicio aeróbico, la temperatura cutánea (Tsk) juega un rol fundamental en la regulación de la temperatura corporal. La temperatura ambiental es el mayor determinante de la Tsk, la cual es el resultado del equilibrio entre la producción metabólica de calor y la disipación de calor hacia el ambiente. Durante el ejercicio, la respuesta de la Tsk puede estar influenciada por el consumo de oxígeno (VO_{2peak}) y por la flexibilidad metabólica, ambos potentes predictores del rendimiento aeróbico. La capacidad elevada de termorregulación cutánea y la tasa de FATox son características en atletas altamente entrenados, mientras que una capacidad oxidativa disminuida, menores valores Tsk y mayor concentración de [La-] son característicos de individuos con una baja capacidad aeróbica, en una misma intensidad de ejercicio sub-máximo absoluto. EL objetivo de este proyecto fue el de analizar y comparar la dinámica de la Tsk en diferentes poblaciones con diferentes respuestas metabólicas durante una prueba de esfuerzo incremental máxima sobre una cinta de correr. Para este propósito se desarrollaron dos estudios. El primer estudio analizó y comparó la correlación entre la Tsk y las variables cardio-respiratorias en varones corredores de fondo con una alta condición física (HF) (n= 35; VO_{2peak} = 56.62 ± 4.31 ml/kg/min) y con una condición física moderada (MF) (n = 44; VO_{2peak} = 47.86 ± 5.29 ml/kg/min) durante un test incremental con periodos de 2 minutos hasta el agotamiento, seguido de un periodo de recuperación de 5 minutos. El segundo estudio analizó y comparó la correlación entre la Tsk y la flexibilidad metabólica midiendo la concentración de [La^-] junto con la tasa de FATox y CHOox corredores de competición altamente entrenados (HT) (n= 22; VO_{2peak} = 58.57 ± 2.33 ml/kg/min), corredores moderadamente activos (MA) (n= 20; VO_{2peak} = 49.07 ± 4.67 ml/kg/min) y jugadores de futbol (SP) (n= 23; VO_{2peak} = 53.34 ± 3.67 ml/kg/min) durante un test máximo incremental con periodos de 3 minutos hasta el agotamiento, seguido de un periodo de recuperación de 5 minutos. Los resultados del

primer estudio mostraron que el grupo MF presentaba menor $VO_{2peak,}$ velocidad máxima ($speed_{peak}$), ventilación (VE) y mayor índice de masa corporal (BMI) y % de masa grasa que el grupo HF (todos $p < 0.001$). La Tsk fue significativamente mayor en el punto de partida, a los 60% y 70% de la carga máxima (todos $p < 0.05$). Los resultados del segundo estudio mostraron que el grupo MA presentaba menor $VO_{2peak,}$ $speed_{peak}$, y mayor BMI y % masa grasa (all $p < 0.05$) que los grupos HT y SP. Existían correlaciones entre Tsk con las tasas de FATox y CHOox y la concentración de [La-] en todos los puntos de datos de todos los grupos (todos $p < 0.001$). Estos resultados indican que el mayor VO_{2peak} y mayor tasa de FATox y menor concentración de [La-] estaban asociadas con un aumento de Tsk durante un ejercicio incremental máximo entre individuos con una amplia diferencia de su capacidad aeróbica. Estas diferencias deberían tenerse en cuenta en las estrategias nutricionales y de entrenamiento para mejorar los deportes aeróbicos y de equipo.

1.3 Versió en Català

Durant l'exercici aeròbic, la temperatura cutània (Tsk) juga un rol fonamental en la regulació de la temperatura corporal. La temperatura ambiental és el major determinant de la Tsk, la qual és el resultat de l'equilibri entre la producció metabòlica de calor i la dissipació de calor cap a l'entorn. Durant l'exercici, la resposta de la Tsk pot estar influenciada pel consum d'oxigen (VO_{2peak}) i per la flexibilitat metabòlica, ambdós potents predictors del rendiment aeròbic. La capacitat elevada de termoregulació cutània i la tassa de FATox són característiques en atletes altament entrenats, mentre que una capacitat oxidativa disminuïda, menors valors de Tsk i major concentració de [La-] són característics d'individus amb una baixa capacitat aeròbica, en una mateixa d'exercici sub-màxim absolut. L'objectiu d'aquest projecte va ser el d'analitzar i comparar la dinàmica de la Tsk en diferents poblacions amb diferents respostes metabòliques durant una proba d esforç incremental màxima sobre una cinta de córrer. Per aquest propòsit es desenvoluparen dos estudis. El primer estudi analitzà i va comparar la correlació entre la Tsk i les variables cardio-respiratòries en homes corredors de fons amb una alta condició física (HF) (n= 35; VO_{2peak} = 56.62 ± 4.31 ml/kg/min) i amb una condició física moderada (MF) (n = 44; VO_{2peak} = 47.86 ± 5.29 ml/kg/min) durant un test

incremental amb períodes de 2 minuts fins l' esgotament, seguit d'un període de recuperació de 5 minuts. El segon estudi analitzà i va comparar la correlació entre la Tsk i la flexibilitat metabòlica mesurant la concentració de [La^-] juntament amb la tassa de FATox i CHOox en corredors de competició altament entrenats (HT) (n= 22; VO_{2peak} = 58.57 ± 2.33 ml/kg/min), corredors moderadament actius (MA) (n= 20; VO_{2peak} = 49.07 ± 4.67 ml/kg/min) i jugadors de futbol (SP) (n= 23; VO_{2peak} = 53.34 ± 3.67 ml/kg/min) durant un test màxim incremental amb períodes de 3 minuts fins l'esgotament, seguit d'un període de recuperació de 5 minuts. Els resultats del primer estudi mostraren que el grup MF presentava menor $VO_{2peak,}$ velocitat màxima ($speed_{peak}$), ventilació (VE) i major índex de massa corporal (BMI) i % de massa grassa que el grup HF (tots $p < 0.001$). La Tsk va ser significativament major en el punt de partida, als 60% i 70% de la càrrega màxima (tots $p < 0.05$). Els resultats del segon estudi mostraren que el grup MA presentava menor $VO_{2peak,}$ $speed_{peak}$, i major BMI i % massa grassa (all $p < 0.05$) que els grups HT y SP. Existien correlacions entre Tsk amb les tasses de FATox i CHOox i la concentració de [La-] en tots els punts de dades de tots els grups (tots $p < 0.001$). Aquests resultats indiquen que el major VO_{2peak} i la major tassa de FATox i menor concentració de [La-] estaven associades amb un augment de Tsk durant un exercici incremental màxim entre individus amb una ampla diferència de la seva capacitat aeròbica. Aquestes diferències haurien de tenir-se en consideració a les estratègies nutricionals i d'entrenament per millorar els esports aeròbics i d'equip.

2. INTRODUCTION

2.1 Introduction

The present PhD thesis and the data analyzed belong to the following research project: *"Thermoregulation in male endurance runners: role of skin temperature during an incremental maximal exercise test".*

The leading researchers of this 4 years long project have been Dr. Miriam Guerra Balic and Dr. Andrea Suárez Segade, supported by the Facultad de Psicología, Ciencias de la Educación y el Deporte de la Blanquerna, Universidad Ramon Llull (Ref.: BRB14-15 SAFE). The project has been developed in collaboration with the Laboratory of Exercise Physiology and Performance Assessment (Labsportsalud) in Cornella (Barcelona). I had the good fortune to participate in an international academic internship with Dr. Bo Fernhall and his team of researchers at their Integrative Physiology Laboratory (IPL) at the University of Illinois at Chicago (UIC), for which this can be considered an international thesis.

In this project, the influence of the skin thermoregulatory response on endurance aerobic performance during an incremental exercise test was studied, where participants were trained endurance runners at different levels of athletic performance, recruited from running and triathlon teams in the Barcelona city area (Spain).

Thermoregulation is key for controlling fatigue and regulating physiological homeostasis during rest and physical exercise. Exercise disturbs the maintenance of body temperature, but humans have the ability to thermoregulate in order to adapt to exercise demands and minimize changes in body core temperature (Tc), and maintain physiological homeostasis (Romanovsky, 2006). Changes in temperature can be perceived at different areas of the human body, where the most critical are the skin, muscle, and core tissues (i.e., of the rectal, visceral, and esophageal) (Kenny & McGinn, 2016). Skin temperature (Tsk) plays a fundamental role in body temperature (BT) regulation, providing negative and positive auxiliary feedback to the thermoregulation system (Romanovsky, 2014).

Measuring Tsk provides important information about the complex thermal control system when studying thermoregulation. Skin temperature is a key factor in the thermoregulatory process, as it is the result of the balance between metabolic heat production and heat dissipation to the environment (José González-Alonso, 2012). This balance is influenced mainly by the responses of Tc, environment temperature (Ta) and complex relationships between cutaneous vasodilation and sweating, which implies that Tsk can be used as an index to predict thermal changes during exercise (H. Liu et al., 2014; Takada, Matsumoto, & Matsushita, 2013).

It has been scientifically demonstrated that changes in skin thermoregulatory capacity may have important physiological implications and substantially influence aerobic exercise performance, and can limit the maintenance of thermal homeostasis during exercise (Charkoudian, 2003; Cuddy, Buller, Hailes, & Ruby, 2013; José González-Alonso, Calbet, & Nielsen, 1999a; Pierzga, Frymoyer, & Kenney, 2003).

Despite these discoveries, Tsk response remains unstudied as an independent parameter in the control of BT during exercise. Tsk response could provide insight into the behavior of the thermoregulatory system during aerobic exercise. Furthermore, it is unclear if the level of aerobic fitness shown as maximal oxygen consumption (VO_{2peak}) influences Tsk response and other metabolic responses, such as metabolic flexibility, during exercise.

Regarding the influence of skin thermoregulatory response on athletic performance, there is a lack of literature reporting the Tsk dynamic in endurance runners during a maximal stress test. We believe that studying and comparing Tsk dynamic across healthy populations during an incremental maximal stress running test may lead to a better understanding of the influence the skin thermoregulatory response has on aerobic performance.

To the best of our knowledge, the dynamic of the skin thermoregulatory response during a maximal stress exercise test and its association with other physiological variables has not been directly studied. Therefore, the main objective of the present study was to analyze and compare the Tsk dynamic and its correlation with other physiological variables across healthy populations with different metabolic

characteristics during an incremental maximal running exercise test. Two separate studies were conducted.

In the first study, the main aim was to analyze and compare the Tsk dynamic in highly (HF) and moderately (MF) fit endurance runners during an incremental maximal stress running test. Furthermore, we analyzed the correlations between Tsk and cardiorespiratory variables.

In the second study, the aim was to analyze the relationships between Tsk and metabolic flexibility (measurements of [La^-], and FAT and CHO oxidation rates) in highly trained (HT) competitive endurance runners, moderately active (MA) runners and professional soccer (PS) players during an incremental maximal stress treadmill test.

In both studies, a recovery period was objectively monitored for any changes that might take place after exercise, which could indicate a relationship between thermoregulatory and physiological responses in endurance runners.

During the development of the study, thanks to the collaboration of various teams and participants, we had the support needed to perform all the proposed tests independent of each participant's level of athletic performance.

2.2 PhD Thesis Development

This thesis is divided into seven parts. The **Introduction** explains and justifies the present study. The **Literature review** is where different concepts relevant to the present thesis are presented, such as the role of Tsk in thermoregulation, Tsk response to exercise, and the relationship between Tsk and both cardiorespiratory and metabolic responses related to VO_{2peak}. Relevant studies are also discussed. In **Aims and hypothesis**, we present our hypothesis based on the literature review and goals for the study are presented. The PhD project is divided into two separate studies. In **Material and methods**, the equipment, protocols and procedures are described, as well as the field and laboratory tests used. Next, the **Results** of the different tests are presented. In **Discussion,** we present the main findings obtained from the study, and the study's limitations. Finally, in **Conclusions** the most relevant results from this thesis are summarized and future research directions are presented.

3. LITERATURE REVIEW

3.1 Thermoregulation

Thermoregulation is defined as the body's ability to maintain BT within certain values, even when the Ta is very different. The regulation of BT is a key factor in controlling fatigue and ensuring the proper functioning of the body's homeostasis process (Romanovsky, 2006). Therefore, knowledge of BT regulation is necessary for understanding the basic concept of "*homeostasis*" and for a wide variety of physiological and clinical applications (Etain et al., 2015). Gagge (1967) concluded that regulation of BT is one of the most important examples of homeostasis, which influences the maintenance and balance of internal temperature (Maté et al., 2007). Maintenance of BT within narrow limits is a major homeostatic function critical for survival (Sessler, 2009).

3.2 Human Thermoregulation and the Environment

A warm body is one of the primary conditions of life. Humans have the physiological, intellectual, and cultural ability to maintain feasible body temperatures under several conditions. The ability to feel and regulate BT is a key feature of human survival. As in other mammals, the thermoregulation process in humans is an important aspect of homeostasis: a state of dynamic stability in an organism's internal conditions, maintained separately from thermal equilibrium with its environment. Different strategies to regulate BT are used to maintain physiological homeostasis.

Humans are homeothermic organisms that regulate and maintain the temperature of the Tc at a constant level regardless of external conditions and the level of motor activity (Romanovsky, 2006). This is achieved by controlling body heat loss and heat gain via autonomic and behavioral thermoeffectors so that heat balance can be achieved.

Our body is our principal source of heat, as opposed to ectothermic animals that receive heat primarily from the environment. For instance, ectotherms rely on environmental conditions to heat up before they can perform vital actions such as foraging or reproducing. Endotherms, in turn, are less dependent on environmental conditions and can therefore live in a wider range of climates, thereby reducing

competition for resources and increasing chances of survival. Our organism generates heat internally to regulate BT through a balance of heat production, absorption, and loss (Cheuvront & Haymes, 2001). Nevertheless, maintaining a stable BT comes at a cost, as endotherms maintain higher energy expenditures than ectotherms with the same body mass. In general, humans are most productive and comfortable in thermoneutral conditions (Schellen et al., 2010).

Human beings are warm-blooded animals, which means that they are able to maintain their vital organs at a stable temperature despite large fluctuations in environmental conditions. Humans do this by carefully balancing heat production and heat loss in a cold or hot environment, which is shown in Figure 1, which is a modification by Ashof and Weber (1958). If more heat is lost than produced, BT will drop, and viceversa, if more heat is produced than lost, body temperature will increase (Sessler, 2009). Control of this heat balance is referred to as thermoregulation and is effectively executed by modulating behavior (e.g. changing clothes) and physiological mechanisms (e.g. shivering or sweating). However, continual presence in thermoneutral conditions is not necessarily healthy. In modern western societies, people tend to live most of their life in buildings where conditions are kept stable and within the thermoneutral zone. Hence, the body has to use fewer resources to defend BT against thermal challenges. What's more, there are indications that reduced exposure to thermal challenges reduces the capacity to cope with thermal challenges in general (Ely et al., 2009). Thermoregulation thus seems to fit the "use it or lose it" paradigm.

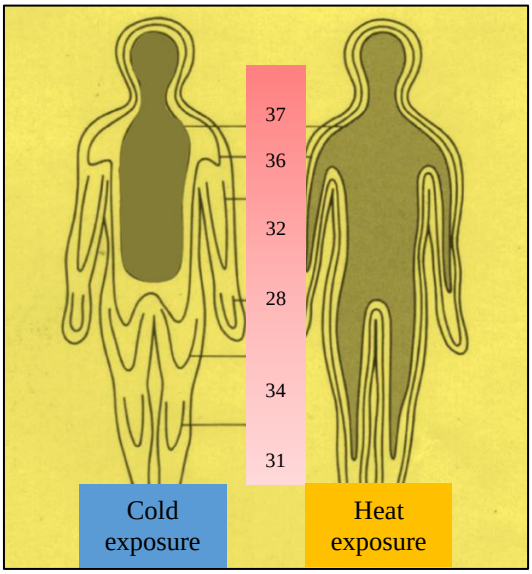

Figure 1: Distribution of temperature during cold or hot exposure in a healthy resting man (adapted by Ashof & Weber, 1958)

Humans possess an elaborate set of mechanisms which regulate BT, which can be broken down into two major processes or control systems: behavioral processes (maintaining or searching for a preferable environment) and autonomic processes (e.g. vasodilation of the skin, sweating and shivering). Therefore, thermal homeostasis is controlled by both behavioral and physiological responses (Table 1).

A powerful form of human thermoregulation is behavioral, examples of such being putting on or taking off clothes, changing posture, moving, taking shelter, etc. Behavioral temperature regulation operates largely through conscious, willed behavior to employ any means available. Accordingly, although frequently overlooked as a physiological variable, behavioral temperature regulation is a crucial form of thermoregulation during both rest and exercise. The human body also has a physiological system of thermoregulation, which operates through the autonomic nervous system, and includes control of: a) the rate of metabolic heat production (i.e., shivering), b) the vasomotor function (i.e., heat flow via the blood from the core to the skin), and c) the sudomotor function (i.e., sweating). Both systems constantly interact with and respond to the changing environment in an attempt to ensure survival and comfort (Parsons, 2003).

Table 1. Key points of Body Temperature (adapted from Schlader & Vargas, 2019)

Body temperature is regulated via both autonomic and behavioral responses.
Current evidence indicates that thermal behavior decreases the requirement for autonomic system responses, so autonomic response activation may contribute to decisions to behaviorally thermoregulate.
Thermal behavior requires an increase in subjective thermal discomfort, the likes of which are perceived from afferent feedback stemming from thermoreceptor activation.
Thus, the autonomic response, manifested in changes in Tsk (occurring secondary to changes in SkBF), skin wettedness (occurring secondary to sweating), or shivering, is consciously perceived to magnify perceptions of thermal discomfort and stimulate thermal behavior.

3.3 Human Behavioral Thermoregulation

Understanding the complex interactions amongst biological, psychological, and environmental factors in homoeothermic organisms represents the primary goal of behavioral thermoregulatory research, and has been the focus of many studies to date. Much progress has recently been achieved since behavioral thermoregulation (thermo-behavior) has become a topic of considerable interest. This field of study has served as a link between thermal physiology, psychology, neurophysiology, and other scientific

areas. Humans play a fundamental role in their own thermoregulation, responding to temperature changes with voluntary responses such as changing PA level, ingesting different types of food, contact with chemical substances, and seeking protection.

Thermoregulatory behavior is a coordinated action that establishes an optimal condition for heat exchange between the environment and the body, and, depending on the circumstance, could entail heat loss, heat gain, or heat balance (IUPS Thermal Commission, 2001). Thermoregulatory behavior is not limited to adjusting the external environment. Voluntary increases in metabolic heat production can also serve as thermoregulatory behaviors acting to achieve thermal comfort. Thermal perception and sense play pivotal roles in behavioral thermoregulation. Notably, the mammalian upper brainstem is known to control emotional whole-body homeostatic behaviors, including behavioral thermoregulation (Flouris & Schlader, 2015a; Schlader et al., 2011; Schlader et al., 2010). The recent attention directed at behavioral thermoregulation stems from the aforementioned evolution in this field as well as the realization of its significance in human thermoregulation. Indeed, thermal homeostasis in humans and other homoeothermic organisms is based primarily on behavioral and secondarily on autonomic and endocrine mechanisms (Flouris & Schlader, 2015b). This is because the latter mechanisms have a finite capacity in preventing hyper/hypothermia, whereas behavioral thermoregulation is a very powerful mechanism that triggers conscious actions that preserve thermal balance when possible (e.g., seeking shade in a hot environment) (Attia, 1984).

3.4 Physiology of Human Thermoregulation

Our thermoregulatory center receives input from two sets of thermoreceptors within the peripheral and central nervous system. Regulation of BT takes place in a hierarchical order. Local mechanisms are at the bottom and central mechanisms are at the top (Guyton, 2000). In general, central thermoregulation is composed of three major components, namely: 1) thermal reception by temperature sensitive neurons, 2) integration through neural pathways and 3) the effective thermoregulatory response through separate branches of the nervous system (Figure 2). In central thermoregulation, information from the entire body is used to decide on the response, whereas in local thermoregulation only local information is used. Thermoregulatory control is dependent

on thermal stimuli from both skin and core (Pitoni et al., 2011). Figure 2 shows a schematic view of human thermoregulation, in which temperature is sensed by neurons that send the afferent information to the hypothalamus, where the information is integrated and the appropriate efferent response is stimulated through separate nervous systems (depicted on the lines). Core temperature cold sensitive neurons are scarce because of low numbers in deep body tissues.

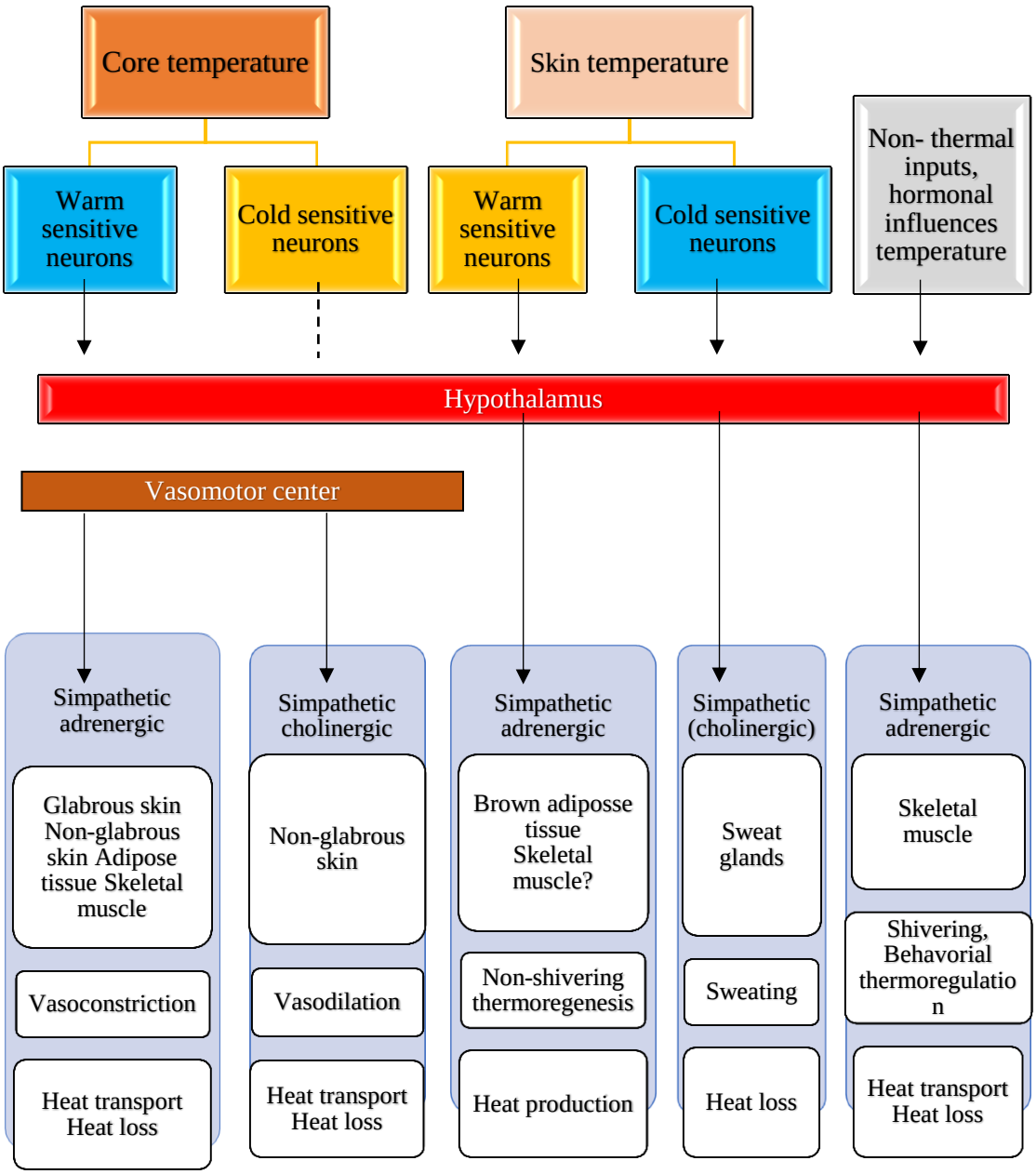

Figure 2: Schematic view of human thermoregulation (adapted by Etain & Johnson, 2015).

3.4.1 Heat Conservation and Dissipation: central and peripheral compartments

Thermally, the human body is considered to be a two-compartment model: a central or core compartment and a cooler peripheral one (Figure 2). When discussing body temperature, we usually refer to the central core (Tc) and peripheral shell temperatures (Ts). In a state of relative physiological rest, BT varies between 36–37.5ºC (Etain et al., 2015). Body temperature is not homogeneous and is described as core body temperature (Tc), skin temperature (Tsk) and mean skin temperature (Tskm). Tc is the most reliable parameter for describing human thermal status and reflects the temperature within the "deep" body tissues and organs that have a high level of basal metabolism (such as the brain, heart, and liver). The strong association between Tc and physiological homeostasis and disturbances makes Tc an important clinical and laboratory indicator of thermal strain in the body. Core temperature is an important indicator of heat strain status and is usually measured using rectal or esophageal probes or ingested telemetry pills (Xu et al., 2013).

Instead, the shell temperature (Ts) refers to the temperatures of the skin, subcutaneous tissue and muscles (Lim et al., 2008). Tsk is used to evaluate local vasoconstriction and Tskm is used to calculate cutaneous heat loss and to estimate central thermoregulatory control (Pitoni et al., 2011). The peripheral tissues – skin and subcutaneous fat, particularly of the arms and legs – are cooler and act as insulation for the core compartment. Under normal thermally comfortable conditions, the temperature of peripheral tissues is about 5–6 ºC lower than the central compartment. Heat is transferred from the central to the peripheral compartments, or conserved in the central compartment, via circulation. A cold stimulus leads to peripheral vasoconstriction, mediated by the sympathetic nervous system, and heat conservation, while a warm stimulus leads to peripheral vasodilatation and heat loss. In extreme environmental temperatures, Tsk may approach central temperature in heat, or 0ºC in the cold. Blood flow and heat loss through the periphery is enhanced by the presence of arteriovenous anastomoses which increases flow and thus enhances heat loss via superficial veins in the skin. In the cold, deep veins, which run alongside the arteries supplying the limbs, take heat from the warm arterial blood supplying the extremities, thus leaving the extremities cold but conserving body heat as an example of a 'countercurrent' mechanism (Campbell, 2011).

The complex interactions between metabolic heat production, the physical properties of skin, the environment, and body size are the principal components determining Tc responses (Figure 3), and ultimately determine whether heat balance is attainable or if Tc will progressively rise to levels that are potentially harmful to health and performance.

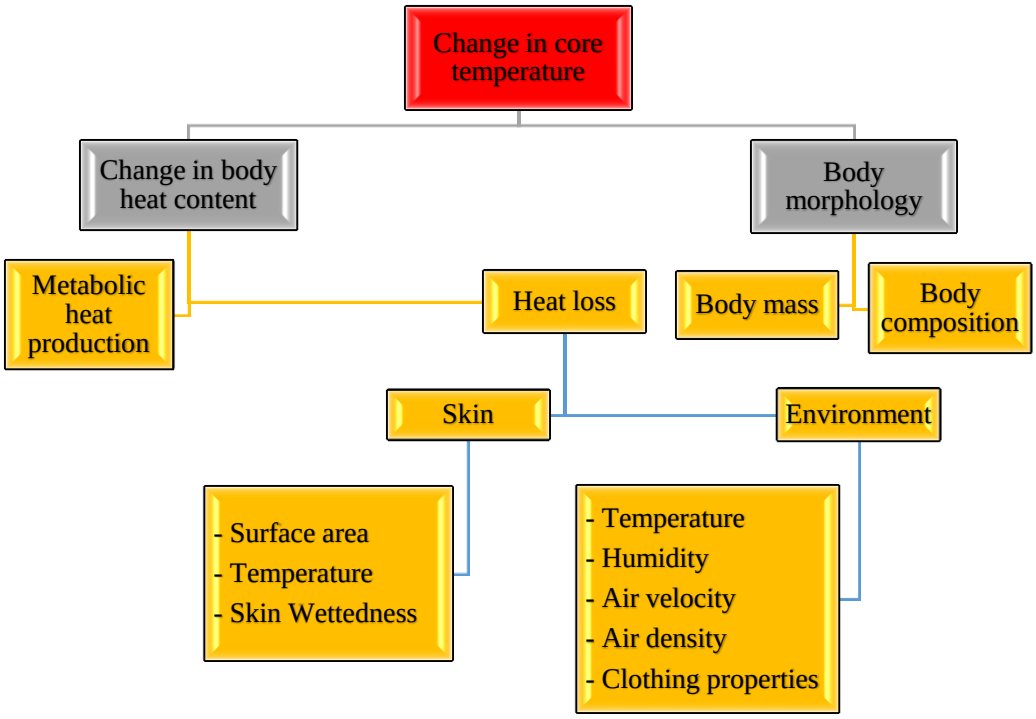

Figure 3: Biophysical factors affecting the change in Tc during exercise and environmental heat exposure (adapted by Cramer & Jay, 2016)

3.4.2 Components of Human Heat Balance

In humans, normal thermoregulation involves a dynamic balance between heat production/gain and heat loss, thereby minimizing any heat exchange with the environment. Everyday activities require a functioning thermoregulatory system that will activate and suppress the heat-dissipating mechanisms to minimize changes in body core temperature. Therefore, we need to understand that heat balance is one of the pillars of successful thermoregulation. Under resting, fasting and thermoneutral conditions total heat production is attributed to the chemical heat released by metabolic processes necessary for keeping our bodies functioning (Parsons, 2003).

A fundamental and useful reference point for any discussion of human energy exchange and Tc regulation is the conceptual heat balance equation:

$$S = M - Wk \pm K \pm R \pm C \pm C_{res} - E_{res} - E_{sk}\ [W].$$ (Cramer & Jay, 2016)

In accordance with the law of energy conservation, the rate of body heat storage (S) is equal to the difference between the rates of metabolic energy expenditure (or metabolic rate, M), external work (Wk), dry heat exchange from the skin by conduction (K), radiation (R), convection (C), convective heat exchange (C_{res}) and evaporative heat loss (E_{res}) from the respiratory tract, and evaporative heat loss from the skin (E_{sk}). The SI unit for rates of energy conversion is watts (W); however, heat balance parameters are often expressed per square meter (W/m^2) of total body surface area (A_D), which is conventionally estimated from body mass and standing height (Cramer & Jay, 2016). It may also be useful to express these values per kilogram of total body mass (W/kg) for certain applications discussed below. In some contexts, metabolic rate is expressed in kilojoules per minute (1 kJ/min ≈ 17 W), kilocalories per minute (1 kcal/ min ≈ 70 W), or metabolic equivalents (1 MET = 58.2 W/m^2).

Metabolism always represents a source of heat gain; dry heat average can lead to heat gain or loss depending on the temperature gradient between the skin and environment, but heat can only be lost by evaporation from the respiratory tract and skin. To maintain heat balance (S=0), the rate of total heat gain from metabolic and environmental heat sources must be equivalent to the rate of total heat loss. It follows that heat storage and internal temperature rises if the rate of total heat gain exceeds the rate of total heat loss ($S > 0$); conversely, heat storage and internal temperature fall if the rate of total heat loss outweighs the rate of heat gain ($S < 0$). The change in body heat content (i.e., change in thermal energy) in kilojoules is the product of time and the net difference between the rates of heat gain and loss. Thus, a constant Tc is maintained through a cascade of reflex vasomotor and cardiovascular responses which correct temperature change by heat loss or heat production (Johnson et al., 2014).

Heat Production

Heat production is counterbalanced through heat exchange with the environment. In general, the thermal environment is colder than our body surface. In that case, all heat exchange with the environment is in fact heat loss. The core consists of the brain and the abdominal and thoracic cavities, which contain the larger organs that under resting conditions are the main producers of heat. There are a limited number of ways to produce heat or increase heat production (Table 2).

Non-shivering thermogenesis (NST) likely takes place in brown adipose tissue and skeletal muscle (Lim et al., 2008). Specialized proteins in the cell mitochondria of these tissues enable the cell to reduce the efficiency of ATP production and release more heat. The increase in heat production by NST is around +5% to +20% of normal heat production (Smith & Johnson, 2016). Several types of heat exchange can be discriminated.

Table 2: Mechanisms and different ways to produce heat, or increase his heat production

Mechanism	Effect
Basal metabolism	Minimum heat production (metabolism) for maintenance of life. Magnitude depends on body size, age and sex.
Muscle contraction	Shivering and voluntary (behavioral) activity.
Dietary-induced thermogenesis	Heat production rises by 10-15% following nutrient (food) intake. Particularly marked with protein.
Non-shivering thermogenesis	Hibernating animals and neonates. Sympathetic stimulation of 'uncoupled' mitochondria in brown adipose tissue.
Hormonal	Thyroid controls overall basal metabolism. Catecholamines increase heat production by stimulation of various metabolic pathways.

Heat Loss

Thermoregulation aims to reduce excessive heat produced by internal organs (Schellen et al., 2010), allowing exercise to be sustained longer (Mattern et al., 2008) and minimizing the risk of medical problems (Nybo, 2008). Heat transfer between the body and the external environment occurs through the processes of radiation, conduction, convection and evaporation, which are the four avenues of heat loss (Figure 4).

The following heat balance equation addresses the internal and external factors that contribute to thermal balance and, therefore, the maintenance of Tc (Etain et al., 2015):

Heat storage = metabolism – work – evaporation ± radiation ± conduction ±convection

where

- **Metabolism** refers to the chemical reactions occurring within the body that produce heat. During exercise, the working muscle liberates large amounts of heat.
- **Work** is the external work done.
- **Evaporation** is the heat loss to environment as water vaporized from the respiratory passages and skin surface. Total sweat vaporized from skin depends on the following three factors:

1. The surface area exposed to the environment
2. The temperature and relative humidity of environment air
3. Convective air currents around the body

Evaporation is the most efficient way to lose heat during exercise, and the ability to thermoregulate is crucial for physical activity.

- **Radiation** is the electromagnetic radiation (heat) transferred to bodies not in physical contact, including the ultraviolet light radiation from the sun, which penetrates through to the surface of the earth, and the infrared radiation of the body. This is the main method for removing heat from the body at rest.

- **Conduction** is the movement of heat to/from the body directly to objects in contact with the body. Usually the amount of heat exchanged in this way is minimal, for example heat lost by our feet to the ground is a form of conduction.

- **Convection** is the transfer of heat to a moving gas or liquid. When body is warm, the air molecules in contact with the body will be warmed,

reducing their density and causing the molecules to rise and be replaced with cooler air. Convective heat exchange is increased by movement of the body in air or water, or movement of air or water across the skin. Conduction and convection are difficult to separate from each other.

Heat transfer through convection, conduction and radiation is bidirectional, where heat transfer between the skin surface and the environment is driven by the temperature gradient between the skin and the surrounding environment. Unlike the other avenues of heat exchange, heat dissipation through evaporation is unidirectional, where heat is transferred only from the skin surface to the external environment (Cheuvront & Haymes, 2001). These components are often lumped together and when heat storage is zero, the body is thermally balanced.

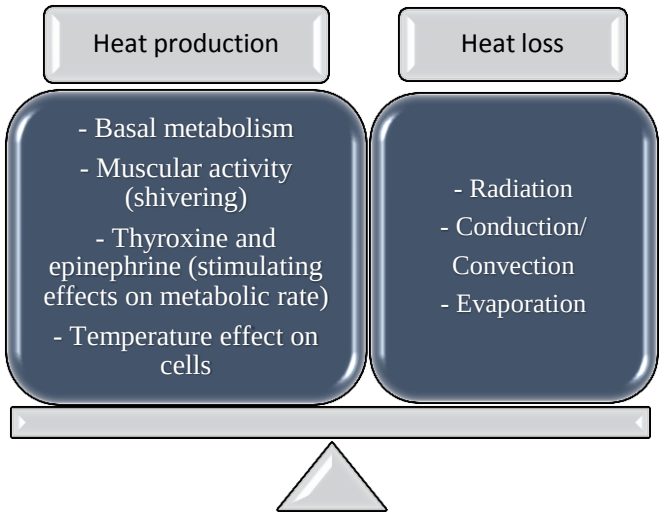

Figure 4: Regulation of body temperature (heat production and heat loss)

3.4.3 Role of the Hypothalamus

Body temperature is controlled by the hypothalamus, which determines the temperature value at which it is to be regulated – the 'set point'. The hypothalamus is the coordinating or central integration center for thermoregulation and sends impulses to several different effectors to adjust BT, allowing the mechanisms of production and heat loss to balance (Figure 5). It is in a region of the brain that links the endocrine system to the nervous system and is therefore an important neural structure with the highest level of thermoregulatory integration (Wendt et al., 2007). This hypothalamic area is composed of the *preoptic-anterior hypothalamus nucleus* (NPHA), which is the

thermostat center of excellence, dorsomedial nucleus of the hypothalamus, and the periaqueductal gray or raphe nuclei (Boulant, 2000). Evidence suggests that the NPHA is the most important region for autonomic temperature control (Etain et al., 2015). The NPHA coordinates all thermoregulatory responses hierarchically and receives afferent sensory input from thermoreceptors throughout the body, including the spinal cord, abdominal viscera, the greater veins and skin. The preoptic area of the hypothalamus contains heat- and cold-sensitive neurons that respond to changes in blood temperature, although heat-sensitive neurons far outnumber cold-sensitive sensors. In this way, the thermosensitive neurons in the hypothalamus are able to initiate the thermoregulatory response most appropriate for any given thermal stress (Wendt et al., 2007). The critical threshold temperature in the hypothalamus, above or below which processes are initiated to increase heat production or heat loss, is ≈ 37ºC and the temperature control mechanisms tend to bring the BT back to the 'set-point' (Benzinger TH., 1969). This regulated level of Tc varies ≈ 1ºC as a result of the BT circadian rhythm. For women, the menstrual cycle also has an influence and BT distribution also varies (Pitoni et al., 2011; Sessler, 2009).

Thus, thermoregulation is an example of the integrative role of the hypothalamus in generating patterns of autonomic, endocrine, motor and behavioral responses to adapt to environmental challenges. Experimental studies have provided new information on mechanisms of thermal sensation, hypothalamic integration, and central effect or pathways involved in thermoregulation (Campbell, 2011). Moreover, experimental evidence indicates that the medial NPHA area, the dorsomedial nucleus of the hypothalamus, the periaqueductal gray matter of the midbrain and the nucleus raphe pallidus in the medulla play a critical role in thermoregulation (Romanovsky, 2006). Deviation from resting BT affects various physiological systems in the body, which dictates the duration of biological functions and dysfunctions that interact with the thermoregulatory mechanisms (Lim et al., 2008). Communication between neural centers within the hypothalamus and peripheral sensors and effector organs regulate, almost exclusively, the processes that maintain stable Tc in homeotherms (Mcdonald, 2009).

We mentioned above that the hypothalamus receives inputs from central and peripheral temperature receptors situated in the ‘core’ and the outer ‘shell’. Peripheral

skin receptors (especially on the trunk) regulate heat loss and transmit temperature information from our skin and the Ta (Sessler, 2009). The mechanism by which temperature is sensed is largely dependent on a temperature-specific family of transient receptor potential (TRP) ion channels (Güler et al., 2002; Romanovsky, 2006). Cutaneous thermoreception is sensed by the TRP family of cation channels, widely expressed in sensory neurons (Pitoni et al., 2011). A subtype, the TRPM8 is activated when environmental temperature is below 27ºC, sensing modest cooling. Afferent signals ascend via thermosensory neurons through pathways such as the spinothalamocortical tract and lateral parabrachial neurons. They are integrated at various levels: the spinal cord, brainstem and hypothalamus. They respond to temperatures between 30ºC and 42ºC, whereas peripheral receptors in the skin respond selectively to either cold or hot stimuli. Cold receptors are present in greater numbers than warm ones, and both are transmitted to the hypothalamus via the spinal cord. The heat receptors respond maximally to a skin temperature of 44ºC and the cold ones to a skin temperature of 25ºC. The cold signals activate the lateral parabrachial nucleus neurons, which promote excitatory inputs to drive Gamma-Aminobutyric acid (GABA) interneurons to inhibit other inhibitory output neurons in the medial preoptic subregions of the preoptic area. These results in a disinhibition of thermogenesis-promoting neurons in dorsomedial hypothalamus and the rostral ventromedial medulla. These fibers activate spinal sympathetic and somatic motor circuits to increase thermogenesis (Flouris & Schlader, 2015b).

There are upper and lower thresholds for hypothalamic temperature, above and below which thermoregulatory mechanisms come into play to conserve/generate or dissipate heat. These upper and lower limits delineate the so called ‘thermoneutral zone’. The thermoneutral zone is a range of temperatures over which a naked individual can maintain body temperature by simply altering vascular tone. These vary with circadian rhythm, sex, exercise, etc., but vasoconstrictive responses are initiated at around 36.5ºC and shivering at 36–36.2ºC.

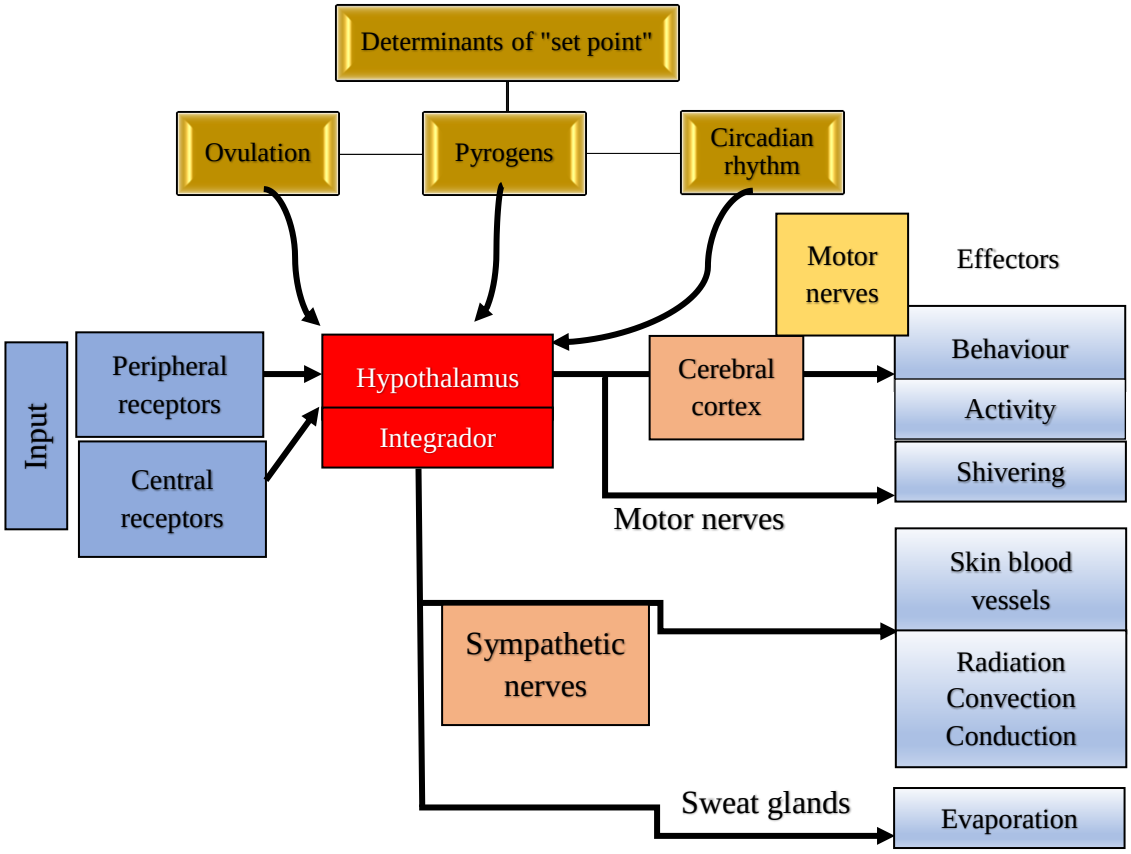

Figure 5: Thermoregulatory mechanisms, an estimate based on the concept of control engineering (adapted by Campbell, 2011)

3.4.4 Temperature Receptors and the Control of Thermoregulatory Mechanisms

Temperature regulation in humans is achieved via autonomic and behavioral thermoeffectors. Autonomic thermoeffectors promote heat loss, heat conservation, or heat gain (Figure 6).

Autonomic responses are governed by inputs sensed by core and surface receptors. Changes in temperature can be perceived at various levels of body tissue with the most critical areas being the skin, muscle, and core (i.e., of the rectal, visceral, and esophageal) tissues. The central thermoregulatory system provides a proportional output that is influenced by both internal and whole-body skin temperatures. Per degree increase above thermoneutral values, the internal temperature is about 10 times more important than mean Tsk in eliciting an output to the sweat glands (Nagashima et al., 2012).

In humans, Tc is maintained in a narrow range known as the interthreshold range (Pitoni et al., 2011), which is around 37°C (98.6°F), and controlled within a narrow range (33.2–38.2°C) to preserve normal physiological function. Under normal physiological conditions temperature can only increase or decrease by a few tenths of a degree Celsius without reaching threshold triggering autonomic thermoregulatory responses (sweating or shivering), (Sessler, 2009). No person has exactly the same temperature at every moment of the day. There is a nearly 1ºC sinusoidal circadian variation around this temperature, with the maximum occurring mid-afternoon and the minimum 12 hrs later (Sessler, 2009). The BT of a healthy person varies during the day by about 0,5°C (0,9°F) with lower temperatures in the morning and higher temperatures in the late afternoon and evening, as the body's needs and activities change (Kelly, 2007). It is interesting that Tc, at any given time, is regulated to within just a few tenths of a degree centigrade, but that the daily variation is much higher. For instance, BT also changes when a person is hungry, sleepy, sick, or cold. In humans, the entire sweating-to-shivering range spans only approximately 0.68ºC (Sessler & Lee, 1991). All this thermal information from the skin surface, peripheral tissues, core organs, and the neuraxis per se are integrated at various levels, finally arriving at the thermoregulatory controller in the hypothalamus (Sessler, 2009).

The dynamics of heat flux during sustained exercise can be briefly summarized (Rowland, 2008) (Figure 6): heat liberated by contracting muscle fibers is transferred away by surrounding blood flow, resulting in an increase in body Tc. In response, hypothalamic control centers and peripheral receptors trigger compensatory cooling mechanisms, principally by 1) cutaneous vasodilation, augmenting SkBF for convective heat loss to the surrounding air and 2) increasing the rate of sweating via sympathetic cholinergic stimulation to dissipate heat by evaporation at the skin-air interface. The magnitude of convective heat loss is governed by the local skin-air temperature gradient as well as adequacy of cutaneous blood flow. This means of heat dispersal is thus most effective in conditions of moderate environmental temperature, and it becomes less so as Ta rises. Heat loss by evaporation is directly related to both the rate of sweat production and the skin-air water vapor pressure gradient. In high Ta, then, body heat loss is affected primarily through sweating, particularly in conditions of low ambient humidity (Rowland, 2008).

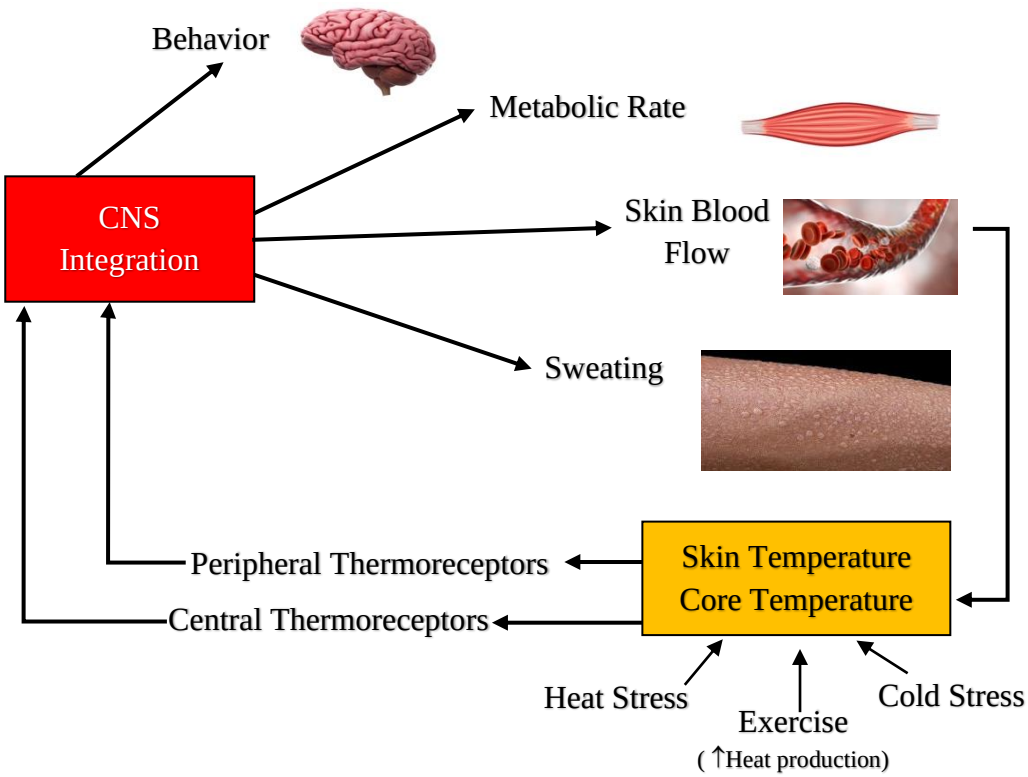

Figure 6: Overview of human temperature regulation as it occurs via both autonomic and behavioral thermoeffectors (adapted by Schlader & Vargas, 2019)

3.5 Skin Temperature: its role in thermoregulation

Skin is a key factor in the homeothermic function of maintaining internal BT. The human body can be separated into an always warm-blooded thermal core and a cold-blooded shell, where the average Tc is 37°C, while the body surface is commonly found to be 33°C. These temperatures depend on a number of variables and are a function of internal organ temperature as well as the thermal properties of the tissues that separate an organ from the surface of the body, including, among others, the muscle tissue and fat content, as well as blood flow, blood temperature, skin moisture and the amount of energy produced during regulated homeostatic metabolic processes (Chudecka & Lubkowska, 2012; Gerrett et al., 2019; Xu et al., 2013).

Human Tsk is an important physiological parameter that reflects the state of heat exchange between the human body and a thermal environment. Romanovsky (2014)

states that Tsk is one of the body's temperatures, and that thermal cutaneous signals serve as feedback signals in the thermoregulation system. Autonomic thermoregulation does not use thermal feedforward signals; all thermal signals used are feedback signals. Thermoregulatory behaviors use similar feedback signals. Overall, the main thermoregulatory role of thermal cutaneous signals is to provide negative and positive auxiliary feedback to the thermoregulatory system, thus reducing the system's response time and making BT more stable (Romanovsky, 2014).

3.5.1 Human Skin

Skin is an essential organ for maintaining Tc within the normal range of 36.1 to 37.8 ºC, which preserves the vital functions of the body (Campbell, 2011). Skin is the largest sensory organ on our body, and serves as a barrier between our internal and external environments and protects the former from diverse unfavourable factors of the latter, thus allowing us to maintain homeostasis (Romanovsky, 2014). Properties of human skin will vary across different areas of the body and will also change with time. In addition to intra-subject differences there will be inter-subject differences. Despite these differences, however, human skin, both between and 'within' humans, has a common structure and most skin is similar in function.

The specific structure of any particular area of skin will depend upon the function of that skin. For example, a vital area for thermoregulation will have a rich blood supply and many sweat glands. Human skin is made up of layers: an outer horny layer of dead cells, the epidermis and the dermis (Figure 7). Under the dermis is a layer of fat: panniculus adiposus. The cutaneous epithelium, at the base of the epidermis, continually generates epidermal cells that move to the surface of the skin, die and eventually are removed from the skin surface (Parsons, 2003).

The function of skin can is to act mainly as a protective and containing barrier while allowing necessary interaction between the body and the environment. Two important interactions are the regulation of heat exchange and the sensory perception of the environment. To aid in performing these functions, human skin contains systems for supporting surface hair and mechanisms for controlling moisture on the skin surface (sweat). These systems are based in the dermis of the skin but penetrate the epidermis to

the surface of the skin. The dermis is also supplied with blood vessels and mechanisms for controlling the lymphatic vessels and nerve receptors which are sensitive to temperature, superficial touch and pressure, and for controlling the flow of blood through them. The skin therefore functions as a dynamic system, changing its condition depending upon the requirements of the body.

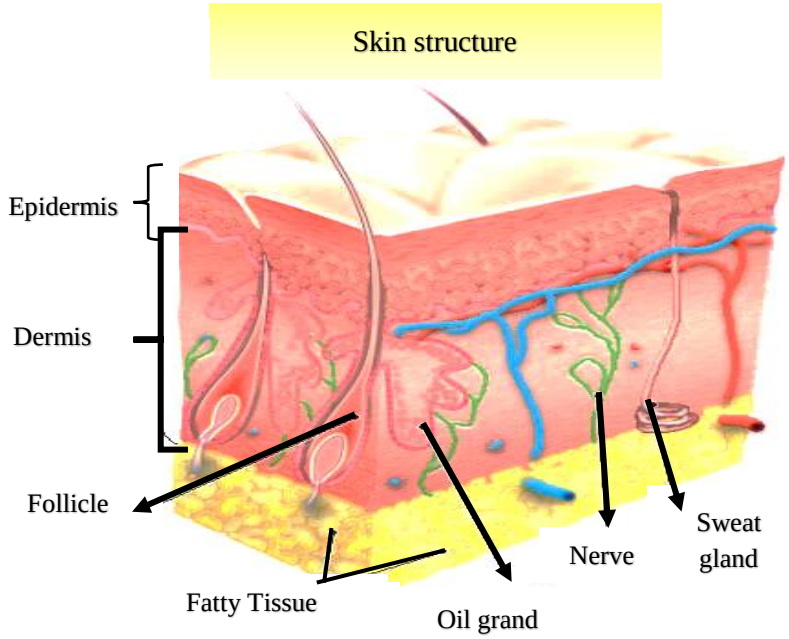

Figure 7: Simple model of human skin

The surface of the human body is a rich map of isotherms with a very wide temperature range that is influenced by endogenous and exogenous changes. The skin contributes to homeostasis by sensing various disturbances occurring at the border of the two environments, including thermal disturbances, and triggering defense responses. But, there is no agreement on which thermal disturbances are detected by the skin, external or internal (Liu et al., 2013).

All body heat loss takes place at the interfaces between the body and the environment. Skin surface accounts for about 92% of total heat loss and the respiratory tract accounts for the remaining 8% (Parsons, 2003). At proximal sites such as the abdomen and thorax, body heat is transported to the skin by conduction from underlying heat producing organs and convection (i.e. blood flow). At distal areas such as the hands, most heat is transported to the skin by blood flow. Skin vasoconstriction reduces

blood flow to the skin. Hence, less heat is transported from the body core to the skin (Romanovsky, 2014).

Reaction of skin to contact with different environmental conditions may depend upon the initial condition of the skin, and may vary in cold conditions where there are low skin temperatures, low blood supply, and 'dry' skin, to hot conditions involving relatively high skin temperatures, rich blood supply and possibly wet skin due to sweating. The above describes the reaction of skin to allow 'normal' thermoregulation. It provides a framework for identifying the possible initial skin condition under which contact with a hot object may occur. Important factors that will affect intra and inter-human variation in skin condition are provided in Table 3 (Wong & Hollowed, 2016).

Table 3: Factors which influence variation in human skin

Factor	**Explanation**
Intra- subject factors	
Area of the body	Regional difference in epithelium structure and thickness; water content; pigmentation
State of vasodilation/vasoconstriction	Instantaneous state of local capillary flood flow
Wet or Dry (e.g. state of thermoregulatory sweating)	Hibernating animals and neonates. Sympathetic stimulation of 'uncoupled' mitochondria in brown adipose tissue.
Inter- subject factors	
Age	Children, adults
Occupation	Use of skin– manual/office worker adults
Sex	Males/females
Ethnic differences	

In humans, an increase in internal Tc elicits large increases in SkBF and sweating. An increase in SkBF serves to transfer heat convection from the body core to the skin surface while sweating results in evaporative cooling of the skin (Ho et al., 1997).

3.5.2 Skin Temperature and Skin Blood Flow

Thermal homeostasis in humans is mainly achieved by regulation of the level of blood flow in the skin. Accordingly, blood perfusion through the vessels in the skin surface constantly adjusts to Tsk (Bruck, 1989) and the heat loss rate changes as a result. If the whole-body becomes 'too hot', blood flows through the dermis (vasodilation) to release heat through the epidermis to the environment. If greater heat loss is required, then the surface of the skin is moistened with sweat so that the latent

heat of vaporization may be lost through evaporation. Under normothermic conditions, blood vessels are under a basal sympathetic tone. Any increase in sympathetic nerve activity induces vasoconstriction, and any decrease in sympathetic nerve activity induces passive vasodilation. Sympathetic adrenergic (vasoconstrictor) nerves innervate both glabrous skin (e.g. lips, forehead, palms and soles) and non-glabrous skin. Therefore, all skin regions are able to modulate SkBF by vasoconstriction or passive vasodilation. Conversely, active vasodilation only occurs in non-glabrous skin, since sympathetic cholinergic (vasodilator) nerves innervate non-glabrous but not glabrous skin. It is not exactly known how neural vasodilation is mediated. However, experiments showed that acetylcholine released by cholinergic nerves is broken down by vessel endothelium (Wendt et al., 2007). During this process, nitric oxide is formed which is a powerful vasodilator. Release of acetylcholine also stimulates the production of sweat from sweat glands (i.e. the sudomotor response). Therefore, the onset of sweating and active vasodilation often coincide. In fact, it has been hypothesized that active vasodilation is actually a side effect of sudomotor control.

During passive heat stress or exercise, the latter of which increases Tc secondarily to increases in metabolic heat production, warm blood is distributed toward the skin via cutaneous vasodilation and subsequent increases in SkBF. When Ta is less than or equal to Tsk, the resulting elevations in Tsk increase the gradient for convective and radiative heat exchange between the skin and the environment. However, when ambient temperature is greater than Tsk, increases in Tsk narrow the temperature gradient, thereby minimizing heat gain from the environment. During cold stress, warm blood is kept in the central circulation via reductions in SkBF that are mediated by cutaneous vasoconstriction. This decreases Tsk, thereby narrowing the temperature gradient between the skin and the environment, which reduces heat loss (Neves et al., 2015).

Changes in SkBF can be elicited by both reflex and local mechanisms, with the former being controlled by the sympathetic nervous system and the latter by local changes in Tsk. Reflex modification of cutaneous vasomotor tone is mediated by both sympathetic vasoconstrictor and active vasodilator systems. The sympathetic vasoconstrictor nerves release norepinephrine that binds with postsynaptic α1- and α2-receptors in the cutaneous vasculature (Smith & Johnson, 2016). Thus, during cold stress, increases in sympathetic vasoconstrictor neural activity promote cutaneous

vasoconstriction. In contrast, during heat stress, decreases in sympathetic vasoconstrictor neural activity promote vasodilation of the cutaneous vascular beds. In addition, further increases in SkBF during heat stress are mediated by active cutaneous vasodilation brought about by activation of the sympathetic vasodilator system. The active vasodilator system is characterized by sympathetic cholinergic nerve transmission, but there are a number of additional important contributors to this response (Johnson et al., 2014). Almost all the increases in SkBF during heat stress are mediated via activation of sympathetic vasodilator nerves (Johnson et al., 2014). Under such circumstances, BP is generally well maintained owing to relatively profound increases in cardiac output (Palombo et al., 2010). The activation of active vasodilation largely corresponds with increases in Tc, whereas the extent to which the cutaneous vasoconstrictor system can modify SkBF is achieved solely through changes in Tsk (Schlader & Vargas, 2019)

In this sense, control of the distribution of blood between the core and skin is explained by applying a concept of control engineering (i.e. feedback system) to the thermoregulation system. In addition, inputs from skin thermal sensors monitoring Ta are involved in a feedforward system, modulating the negative feedback system (Figure 8). This allows the primary regulated variable, Tc, to remain relatively constant under widely varying environmental conditions.

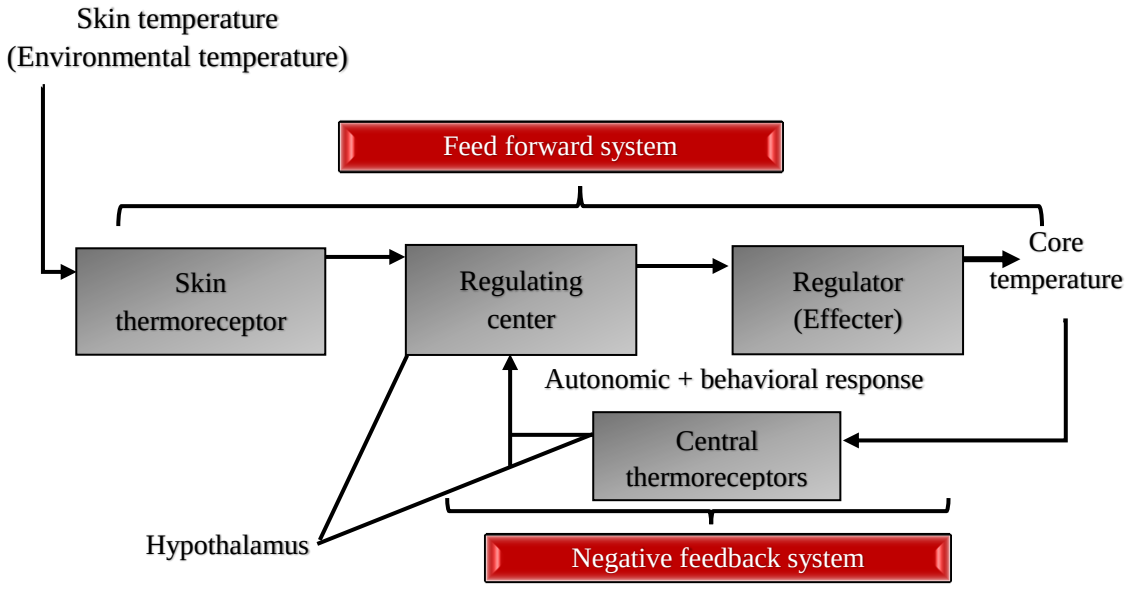

Figure 8: The thermoregulatory system, an estimate based on the concept of control engineering (adapted by Nagashima et al., 2012)

3.5.3 Sweating

Sweating is a powerful mechanism for increasing heat loss; vaporization of 1 ml of sweat removes about 2.4 kJ of heat from the body (Sessler, 2009). Sweating involves the secretion of body fluids onto the surface of the skin, which increases skin wettedness, and evaporative heat loss. Sweating is activated when Tc is increased, but can be influenced by the temperature of the skin (Shibasaki et al., 2010). When the gradient between Tsk and Ta is reduced, heat dissipation via changes in SkBF is minimized. In these instances, heat loss is mostly dependent on sweat evaporation. The rate of evaporative heat loss from the skin (E_{sk}) is driven by the water vapor pressure gradient between the skin and the environment air. Environments with high absolute humidity reduce the water vapor pressure gradient between the skin and environment air, which can further impede heat loss. Sweat glands are largely innervated by sympathetic cholinergic nerves. The stimulation of the sweat glands is controlled by the acetylcholine, along with a number of other neurotransmitters, from the cholinergic nerves on the muscarinic receptors on the eccrine sweat gland (Shibasaki et al., 2010). Upon stimulation, the secretory bulbous secretes an isotonic precursor fluid that is similar in osmolality to plasma, but without plasma proteins, into the duct portion of the gland. As this fluid moves through the secretory duct toward the surface of the skin, most of the electrolytes are reabsorbed. Thus, sweat is a hypotonic fluid and hypovolemia caused by prolonged sweating results in a hyperosmotic state (Schlader & Vargas, 2019).

3.6 Respiratory Heat Loss

In addition to skin, heat is also exchanged between the respiratory tract and the external environment during pulmonary ventilation. When a person inspires, air travels down the airway, is heated and fully saturated with moisture drawn from the airway surface. Upon expiration, heat transferred to the inspired air via convection and evaporation is lost to the surrounding environment. The rate of respiratory heat exchange will depend on the temperature gradient between the inspired air and body core, environment humidity, and ventilation rate. Although respiratory heat loss is highest in cold/dry conditions, it contributes marginally to total whole-body heat loss due to the relatively poor thermal conductivity of air (Cramer & Jay, 2016).

3.7 Thermoregulation to Heat Stress

Heat stress causes a transient or persistent imbalance between heat gained and heat lost to the environment, resulting in body heat storage. Heat gain arises as a byproduct of cellular metabolism and/or exposure to external temperatures greater than the body surface. Humans use sweat thermoregulation for body heat removal, particularly to remove the heat produced during exercise. It is commonly recognized that Tc and thermoregulatory sweating responses to heat stress demonstrate a high degree of individual variability that may be explained by a variety of physiological and biophysical factors (Périard et al., 2014).

The effects of biological maturation on thermoregulatory responses to exercise in hot ambient conditions have been well documented (Campbell, 2011; Rowland et al., 2008; Xu et al., 2013).

3.8 Exercise Physiology

The physiology of physical exercise (PE) involves the study of acute responses and chronic adaptations to exercise. Exercise is the most common stress experienced by our body and requires the integrated functioning of all body systems (Froelicher, 1993):

- Nervous system is essential for the coordination of the different organs and for the exercise performance.
- Somatic nervous system regulates the musculoskeletal function and allows movement to be performed.
- Autonomic nervous system acts largely unconsciously and regulates bodily functions such as HR, digestion, respiratory rate, pupillary response, blood circulation, urination and sexual arousal. It is regulated by the hypothalamus.
- Skeletal-muscle system regulates the movement.
- Cardiovascular system increases the supply of oxygen and energy substrates. This implies an increase in the blood circulation rate and, consequently, an increase in cardiac output.

- Respiratory system regulates ventilation with changes in respiratory rate, tidal volume and minute respiratory volume in order to maintain the correct gas exchange (O_2 and CO_2).
- Endocrine system is a chemical messenger system comprising feedback loops of hormones released by internal glands of an organism directly into the circulatory system, regulating distant target organs.
- Thermoregulatory system regulates the BT, maintaining internal temperature in a state of equilibrium (homeostasis). This system will conserve the vital functions, and plays a major role in high thermal stress conditions and prolonged events.

Maximal effort may be limited by any of the systems involved. Thus, when there is an increase in workload, it is necessary to provide enough oxygen and other substrates to active muscles, in which the resulting CO_2 must also be eliminated (Caspersen, 1985).

So that muscles can perform more work, the bioenergetic conversion of stored chemical energy, such as glucose or fatty acids, into kinetic energy is needed. The respiratory system ensures that changes in respiratory mechanisms are adequate for maintaining the correct functioning of gas exchange. To meet the needs of peripheral muscles, the supply of oxygen and glucose must be increased, which implies more blood volume and therefore an increase in cardiac output. In individuals with normal peripheral respiratory and muscle function, it seems that the cardiac output limits exercise capacity. The nervous system regulates and controls the different systems during exercise (Despopoulos, 1996).

The physiological adaptation to effort differs for sedentary individuals or physically active ones. Adaptation to effort is significantly modified for individuals with high weight or who are very sedentary, to the point that functional assessment sometimes suggests normal behavior (Despopoulos, 1996).

3.9 Physical Exercise

The term PE refers to "performance of some activity in order to develop or maintain physical fitness and overall health". It is often directed toward also honing athletic ability or skill. The characteristics of the personal and socio-cultural experience (Devís, 2000) must be added to this concept in order to understand why people perform certain activities and not others (López-López, 2008).

This PE may have several aspects or purposes (Shepard, 1994):

- As a useful activity, including job activities and domestic tasks.
- Leisure time activities, mainly ludic and recreational.
- Physical Education, an activity with an educational purpose, which doesn't exclude the previous ones.

When we talk about PE we refer to a subcategory of PA, which is any body movement, structured and repetitive, whose purpose is improving or maintaining fitness level and/or motor capacities and abilities (motor learning). So, PE constitutes a stimulus to the development and improvement of the psycho-physical qualities of people (López-López, 2008).

Movement becomes a PA when it has the following characteristics:

- Willfulness: full conscience activities.
- Intentionality: activities with a clear intention. For education, health, leisure, and other.
- Systematization: activity with a specific order, intensity and difficulty, among other characteristics.

Exercise and exercise training frequently are used interchangeably and generally refer to PA performed during leisure time with the primary purpose of improving or maintaining physical fitness, physical performance, or health (Physical Activity Guidelines Advisory Committee, 2008).

PE can be classified as an aerobic or anaerobic activity depending on the principal metabolic pathways involved to produce energy, and can be of several types,

depending on aerobic endurance, strength (resistance) or balance (Giannuzzi et al., 2003).

The intensity with which PA is performed represents the rhythm or effort level used. To classify intensity, the Metabolic Equivalent of Task (MET) is very commonly used. The MET corresponds to the quantity of energy that the body consumes at rest, and 1 MET is defined as oxygen consumption (VO_2) at rest, which is approximately equivalent to 3.5 ml·Kg-1·min-1. Based on the metabolic units, the intensity of PA can be classified as: low (< 3.00 METs), moderate (3.00 a 5.99 METs), vigorous (6.00 a 8.99 METs) and very vigorous (≥ 9.00 METs) (Ainsworth et al., 2000; Haskell et al., 2007).

Table 4 shows the classification of exercise intensities using relative and absolute methods commonly used in practice. Not all of these methods of measurement for exercise intensity have been compared simultaneously, therefore, it cannot be assumed that one method of determining exercise intensity is necessarily equivalent to that derived using another method. It is prudent to keep in mind that the relationships among actual energy expenditure, HR reserve (HRR), VO_{2R}, percent of maximal HR (%Hr max) and VO_{2max} % can vary considerably depending on exercise test protocol, exercise mode, exercise intensity, resting HR, fitness level, age, body composition, and other factors (American College of Sports Medicine, 2018; Cunha, Midgley, Monteiro, & Farinatti, 2010; Fernhall et al., 2001)

Table 4. Classification of exercise intensity: relative and absolute exercise intensity for cardiorespiratory endurance and resistance exercise (American College of Sports Medicine, 2018)

	Cardiorespiratory Endurance Exercise											**Resistance Exercise**
	Relative Intensity				**Intensity (%VO_{2max}) Relative to Maximal Exercise Capacity in METs**			**Absolute Intensity**	**Absolute Intensity (MET) by Age**			**Relative Intensity**
Intensity	**%HRR or %VO_2 R**	**%HR_{max}**	**%VO_{2max}**	**Perceived exertion (Rating on 6-20 RPE Scale)**	**20 METs %VO_{2max}**	**10 METs %VO_{2max}**	**5 METs %VO_{2max}**	**METs**	**Young (20-39 yr)**	**Middle-aged (40-64)**	**Older (≥ 65 yr)**	**% 1RM**
Very light	<30	<57	<37	<Very light (RPE <9)	<34	<37	<44	<2	<2.4	<2.0	<1.6	<30
Light	30-39	57-63	37-45	Very light-fairly light (REP 9-11)	34-42	37-45	44-51	2.0-2.9	2.4-4.7	2.0-3.9	1.6-3.1	30-49
Moderate	40-59	64-76	46-63	Fairly light to somewhat hard (RPE 12-13)	43-61	46-63	52-67	3.0-5.9	4.8-7.1	4.0-5.9	3.2-4.7	50-69
Vigorous (very hard)	60-89	77-95	64-90	Somewhat hard to very hard (RPE 14-17)	62-90	64-90	68-91	6.0-8.7	7.2-10.1	6.0-8.4	4.8-6.7	70-84
Near-maximal to maximal	≥90	≥96	≥91	≥Very hard (RPE ≥ 18)	≥91	≥91	≥92	≥8.8	≥10.2	≥8.5	≥6.8	≥85

Abbreviations: Hr_{max} (maximal HR); HRR (heart rate reserve); MET (metabolic equivalent) ; RPE (ratings of perceived exertion); VO_{2max} (maximal oxygen uptake); VO_2 R (oxygen uptake reserve); 1RM (one maximal repetition).

In Spain, Chodzko-Zajko et al. (2012) found that 84% of autonomous communities have some kind of recommendations for aerobic PE and 37% do for resistance training. The WHO criteria are: aerobic PA (n = 11; 58%), adults (n = 10; 53%), older adults (n = 5; 26%), childhood and adolescents (n = 1; 5%); resistance training for adults (n = 6; 32%), older adults (n=3; 16%), childhood and adolescents (n=1; 5%); balance (n=5; 26%); bouts of continuous PA for at least 10 minutes (n=6; 32%); recommendation of 300 minutes of PA a week (n=10; 53%); PA intensities (n=2; 11%). Communities with higher aging indexes and higher percentages of children/adolescents mostly don't give recommendations of PA related to the WHO guidelines (World Health Organization, 2010).

Tables 5 and 6 show recommendations for PA by different institutions, and evidence of their influence on health status.

Table 5. Recommendations from different institutions for the practice of PA in adults (ACSM, 2018)

Institution	Document	Year	Recommendation of PA for adults	Other recommendations
Gobierno de Chile	Program of PA for Prevention and Control of Cardiovascular Risk Factors	2004	20 to 60 minutes of moderate-vigorous PA	3 to 5 days a week
Generalitat de Catalunya	Prescription Guidelines of Exercise for Health	2007	≥30 minutes of PA	2 – 5 days a week
WHO	Global recommendations on PA for health	2010	≥150 minutes a week of moderate PA ≥75 minutes a week of vigorous PA	≥5 days a week Bouts ≥10 minutes
Ministerio de Salud de la Presidencia de la Nación	Reference Manual of PA and Health in Argentina	2012	≥150 minutes a week of moderate PA ≥75 minutes a week of vigorous PA	≥5 days a week Bouts ≥10 minutes
ACSM	Quantity and quality of exercise for developing and maintaining cardiorespiratory, musculoskeletal, and neuromotor fitness in apparently healthy adults: guidance for prescribing exercise	2014	≥30 minutes moderate PA ≥20-25 minutes of vigorous PA	≥5 days a week ≥3 days a week

Table 6. Health benefits associated with regular physical activity (US Department of Health and Human Services, 2008)

Children and Adolescents
Strong evidence • Improved cardiorespiratory and muscular fitness • Improved bone health • Improved cardiovascular and metabolic health biomarkers • Favorable body composition Strong evidence • Reduced symptoms of depression
Adults and Older Adults and Adolescents
Strong evidence • Lower risk of early death • Lower risk of coronary heart disease • Lower risk of stroke • Lower risk of high blood pressure • Lower risk of adverse blood lipid profile • Lower risk of type 2 diabetes • Lower risk of metabolic síndrome • Lower risk of colon cáncer • Lower risk of breast cancer • Prevention of weight gain • Weight loss, particularly when combined with reduced calorie intake • Improved cardiorespiratory and muscular fitness • Prevention of falls • Reduced depression • Better cognitive function (for older adults) Moderate to strong evidence • Better functional health (for older adults) • Reduced abdominal obesity Moderate evidence • Lower risk of hip fracture • Lower risk of lung cancer • Lower risk of endometrial cancer • Weight maintenance after weight loss • Increased bone density • Improved sleep quality

3.10 Physical Fitness and Training

Fitness is the energy and vitality status that allow people to perform daily and regular tasks, like enjoying active leisure time, confronting unexpected events without excessive fatigue (Pancorbo Sandoval, 2004).

Physical fitness can be divided into health-related and skill-related components. Health-related fitness includes those components of physical fitness that are most

directly related to good health, decreased morbidity and mortality, and improvement in quality of life and well-being (Departament de Salut i Secretaria General de l'Esport del Departament de la Vicepresidència de la Generalitat de Catalunya, 2007; Ministerio de Salud de la Nación Argentina, 2012; Rimmer, 2000). The Sport Committee of the European Council states that "fitness related to health is composed of the following elements: cardiorespiratory endurance, strength, muscle resistance, flexibility, anthropometric dimensions (body composition), coordination-balance and a good psycho-emotional status" (Pancorbo Sandoval, 2004). Good physical fitness reduces the risk of health problems related to lack of exercise, and establishes a fitness base for participation in a variety of activities.

Different motors skills such as coordination, balance, gait and agility (neuromotor exercise training) are not the main goals for health-related fitness, but the lack of them may in fact be harmful in some cases. Developing these skills should be part of a comprehensive exercise program (ACSM, 2018). A program of regular exercise that includes cardiorespiratory, resistance, flexibility, and neuromotor exercise training beyond activities of daily living for the improvement and maintenance of physical fitness and health is essential for most adults. In addition to exercising regularly, there are health benefits in concurrently reducing total time engaged in sedentary pursuits, and by interspersing frequent, short bouts of standing and PA between periods of sedentary activity, even in physically active adults (ACSM, 2018). Sport training is the planned and complex process of organizing progressively increasing workloads in order to stimulate the physiological process of super-compensation of the organism, facilitating the development of different physical qualities and capacities, to promote and consolidate sport performance (Vicente, 1995).

3.10.1 Cardiorespiratory Fitness

A person's total capacity for physical performance is determined by his/her capacity for aerobic and anaerobic performance. His/her aerobic or cardiorespiratory fitness (CRF) is related to the ability to perform large muscle, dynamic, moderate-to-vigorous intensity exercise for prolonged periods of time (ACSM, 2018). CRF is the ability of the circulatory and respiratory systems to supply oxygen to working muscles

during sustained PA (Chicharro et al., 2006; Physical Activity Guidelines Advisory Committee, 2008). Performance of exercise at this level of physical exertion depends on the integrated physiologic and functional state of the respiratory, cardiovascular, and musculoskeletal systems.

CRF is considered a health-related component of physical fitness because (a) low levels of CRF have been associated with a markedly increased risk of premature death from all causes and specifically from CVD; (b) increases in CRF are associated with a reduction in death from all causes; and (c) high levels of CRF are associated with higher levels of habitual PA, which in turn are associated with many health benefits. Because of that, the assessment of CRF is an important part of any primary or secondary prevention and rehabilitative program.

A sedentary lifestyle and a low CRF are known to be major independent risk factors for cardiovascular diseases (CVDs) and all-cause mortality (U.S. Department of Health and Human Services, 2008; World Health Organization, 2007, 2010). CVDs are the first cause of disability and premature morbidity and mortality throughout the world (World Health Organization, 2007, 2010).

The standard measure to express CRF is VO_{2max} or VO_{2peak}, which provides important information about CRF and is a powerful marker of aerobic performance. It is defined as the maximum amount of oxygen that the body is able to absorb, transport and consume per unit of time and is achieved during a maximal cardiorespiratory exercise test, in which a large muscle mass is used (Chicharro et al., 2006). This variable is typically expressed clinically in relative ($mL \cdot kg^{-1} \cdot min^{-1}$) as opposed to absolute ($mL \cdot min^{-1}$) terms, allowing for meaningful comparisons between/among individuals with differing body weight. VO_{2max} is the product of the maximal cardiac output cardiac output (Q) ($L\ blood \cdot min^{-1}$) and arterial-venous oxygen difference ($mL\ O_2 \cdot L\ blood^{-1}$). Significant variation in VO_{2max} across populations and fitness levels results primarily from differences in Q in individuals without pulmonary disease; therefore, VO_{2max} is closely related to the functional capacity of the heart. The designation of VO_{2max} implies an individual's true physiologic limit has been reached and a plateau in VO_2 may be

observed between the final two work rates of a progressive exercise test. This plateau is rarely observed in individuals with CVD or pulmonary disease. Therefore, peak VO_2 is commonly used to describe CRF in these and other populations with chronic diseases and health conditions (ACSM, 2018).

A variety of submaximal and maximal exercise tests can be used to estimate VO_{2max}. These tests have been validated by examining (a) the correlation between directly measured VO_{2max} and VO_{2max} estimated from physiologic responses to submaximal exercise (e.g., HR at a specified power output); or (b) the correlation between directly measured VO_{2max} and test performance (e.g., time to run 1 or 1.5 mi [1.6 or 2.4 km]), or time to volitional fatigue using a standard graded exercise test protocol. It should be noted that there is the potential for a significant overestimation of directly measured VO_{2max} by these types of indirect measurement techniques.

It must be taken into account that results can be overestimated when (a) the exercise protocol chosen for testing is too aggressive for a given individual (i.e., Bruce treadmill protocol in patients with congestive heart failure (CHF); or (b) when treadmill testing is employed and the individual relies on handrail support (ACSM, 2018).

Aerobic fitness level is an important determinant in the health status of individuals of any age. It was reported that maximum oxygen consumption (VO_{2max}) decreases by about 7% per decade (Wilson & Tanaka, 2000). The highest achieved O_2 during a maximal cardiorespiratory exercise test can be considered as VO_{2max} (a plateau in O_2 with an increase in work rate). Secondary criteria to check whether VO_{2max} is reached without a plateau, at least two of these criteria must be met: (1) a RER >1.1, (2) a plateau in HR with an increase in work rate or within 10 beats of the estimated HR_{max}, (3) no change (increase lower than 150 ml·min-1) in O_2 with an increase in workload, and (4) high levels of lactic acid in the minutes following exercise (Chicharro & Redín, 2006; Reaño & Ricart, 2001; Wilmore & Costill, 2004). VO_{2peak} is the highest achieved oxygen uptake during a test without the VO_{2max} criteria. Oxygen consumption is a marker of metabolic rate, and it is directly proportional to training intensity. Whilst this

measurement may be accurate in the laboratory, it is difficult to measure out of the laboratory during training.

The variability among different subjects is wide and depends on various factors such as: genetic endowment, age (Table 7), body composition, sex, and training levels or fitness conditioning (ACSM, 2018). Genetic factors determine the initial VO_{2max} value observed before any training, as well as the capacity to adapt to training by increasing $VO_{2.}$ This capacity varies between 0 and 50%.

Possible mechanisms that limit VO_{2max} could be both centrals and peripherals. Within the centrals are cardiac output (ACSM, 2018), which is the product of heart rate (HR) and stroke volume (SV). Peripherals factors are mitochondrial mass and capillary density (López Chicharro & Izquierdo Redín, 2006).

Table 7. Cardiorespiratory fitness classifications (VO_{2max}) for men by age (ACSM, 2018)

VO_{2max} ($mL \cdot kg^{-1} \cdot min^{-1}$)						
Percentile		**20-29**	**30-39**	**40-49**	**50-59**	**60-69**
95	Superior	66.3	59.8	55.6	50.7	43.0
90	Excellent	61.8	56.5	52.1	45.6	40.3
85		59.3	54.2	49.3	43.2	38.2
80		57.1	51.6	46.7	41.2	36.1
75	Good	55.2	49.2	45.0	39.7	34.5
70		53.7	48.0	43.9	38.2	32.9
65		52.1	46.6	42.1	36.3	31.6
60		50.2	45.2	40.3	35.1	30.5
55	Fair	49.0	43.8	38.9	33.8	29.1
50		48.0	42.4	37.8	32.6	28.2
45		46.5	41.3	36.7	31.6	27.2
40		44.9	39.6	35.7	30.7	26.6
35	Poor	43.5	38.5	34.6	29.5	25.7
30		41.9	37.4	33.3	28.4	24.6
25		40.1	35.9	31.9	27.1	23.7
20		38.1	34.1	30.5	26.1	22.4
15	Very poor	35.4	32.7	29	24.4	21.2
10		32.1	30.2	26.8	22.8	19.8
5		29.0	27.2	24.2	20.9	17.4
		(*n* = 513)	(n= 963)	(n= 1,327)	(n= 1,078)	(n= 593)

3.11 Physical Fitness Evaluation

Measurement of physical fitness is a common and appropriate practice in research and clinical settings. The purposes of health-related fitness testing in such programs include the following (ACSM, 2018):

- Educating participants about their present health-related fitness status relative to health-related standards and age and sex matched norms.
- Providing data that are helpful in the development of exercise prescriptions to address all fitness components.
- Collecting baseline and follow-up data that allow evaluation of progress by exercise program participants.
- Motivating participants by establishing reasonable and attainable fitness goals.
- Stratifying cardiovascular risk.

3.11.1 Measurement of Breath-by-Breath Respiratory Systems

The measurement of VO_{2max} and carbon VCO_{2max} are standard tools of exercise physiology that are used to assess aerobic capacity, exercise intensity and energy expenditure (EE). In addition, measurement of VO_2 and VCO_2 allows to indirectly measure substrate utilization.

Open circuit spirometry is used to measure VO_{2max}. Modern automated systems provide ease of use and a detailed printout of test results (ACSM, 2018). However, system calibration is still essential to obtain accurate results. Because of costs associated with the equipment, space, and personnel needed to carry out these tests, direct measurement of VO_{2max} is generally reserved for research settings.

3.11.2 Body Composition

Body composition is a common and important element in fitness evaluation. It involves a relative representation of the various constituent elements of total body weight. It is well known that it changes under the influence of continuous PA, and is

one of the major components of fitness and the general health of athletes (Mazić et al., 2014).

Body composition refers to the relative proportions of fat, muscle, bone and residual body mass (BM) of the total weight of a person. All fitness components depend on body composition to some extent. It has been well demonstrated than excess body fat (BF) is bad for health, and the pattern of fat distribution is important. Body composition gives more information than weight alone.

Another simple index of weight-for-height that is commonly used to classify underweight, overweight and obesity in adults is body mass index (BMI). It is defined as weight in kilograms divided by the square of height ($kg \cdot m^{-2}$), and is commonly used as a health indicator. Body composition can be estimated using both laboratory or field techniques (ACSM, 2018); Esparza Ros, 1993; WHO, 2000).

Basic body composition can be expressed as the relative percentage of BM that is fat and fat-free tissue using a two-compartment model. Before collecting data for body composition assessment, the technician must be trained, experienced in the techniques, and already have demonstrated reliability in his or her measurements, independent of the technique being used.

Body composition assessment in elite athletes and everyone who is involved in physical activity is of great importance as a determinant of their performance. Endurance athletes such as distance runners, cyclists, and triathletes benefit greatly from having a low BF %. An increase in lean body mass contributes to strength and power development. Strength and power are related to muscle size. Thus, an increase in lean body mass enables the athlete to generate more force in a specific period of time. A sufficient level of lean BM also contributes to speed, quickness, and agility performance (in the development of force applied to the ground for maximal acceleration and deceleration). Additional weight (nonessential fat) provides greater resistance, forcing the athlete to increase the muscle force of contraction per given workload. The additional body fat can limit endurance, balance, coordination, and movement capacity.

Joint range of motion can be negatively affected by excessive body mass and fat as well, and mass can form a physical barrier to joint movement in a complete range of motion (Table 8).

Table 8. Fitness categories for body composition (% Body Fat) for men by age

		Age (year)					
%		20-29	30-39	40-49	50-59	60-69	70-79
99	Very lean	4.2	7.3	9.5	11.0	11.9	13.6
95		6.4	10.3	12.9	14.8	16.2	15.5
90	Excellent	7.9	12.4	15.0	17	18.1	17.5
85		9.1	13.7	16.4	18.3	19.2	19.0
80		10.5	14.9	17.5	19.4	20.2	20.1
75	Good	11.5	15.9	18.5	20.2	21.0	21.0
70		12.6	16.8	19.3	21.0	21.7	21.6
65		13.8	17.7	20.1	21.7	22.4	22.3
60		14.8	18.4	20.8	22.3	23.0	22.9
55	Fair	15.8	19.2	21.4	23.0	23.6	23.7
50		16.6	20.0	22.1	23.6	24.2	24.1
45		17.5	20.7	22.8	24.2	24.9	24.7
40		18.6	21.6	23.5	24.9	25.6	25.3
35	Poor	19.7	22.4	24.2	25.6	26.4	25.8
30		20.7	23.2	24.9	26.3	27.0	26.5
25		22.0	24.1	25.7	27.1	27.9	27.1
20		23.3	25.1	26.6	28.1	28.8	28.4
15	Very poor	24.9	26.4	27.8	29.2	29.8	29.4
10		26.6	27.8	29.2	30.6	31.2	30.7
5		29.2	30.2	31.3	32.7	33.3	32.9
1		33.4	34.4	35.2	36.4	36.8	37.2
n =		1,844	10,099	15,073	9,255	2,851	522

3.12 Cardiorespiratory Tests

a) Laboratory tests:

Aerobic laboratory tests are used to obtain metabolic data. The use of maximal treadmill/bike tests with open circuit spirometry allows for direct and accurate assessment of VO_2 and anaerobic threshold in sport populations. This test has been shown to be a reliable and feasible test (ACSM, 2018).

a) Body Composition Tests:

Anthropometric methods for body composition assessment are (Mazić et al., 2014):

1. Direct laboratory methods: the values obtained with these techniques are quite

accurate but invasive, so their use is practically unviable; they give us direct values without the necessity of posterior transformation equations (Sirvent Belando & Garrido Chamorro, 2009). These two methods are:

- Body dissection, very difficult and with technical problems.
- Tissue biopsy, but its viability is greatly limited due to the requirement of penetration in human tissues.

1. a) Indirect laboratory tests:

- Hydrodensitometry (Underwater) weighing: this technique of measuring body composition is based on Archimedes' principle related to water displacement. This loss of weight in water allows calculation of body volume. Hydrodensitometry weighing has a high reliability (r = .98 to .99) as reported by Rimmer et al., (1987).

- Dual-energy X-ray absorptiometry (DEXA): these scanners remain relatively straightforward to operate with no need for participant involvement and have reported accurate measurements in diverse populations (Pritchard et al., 1993). The review performed by Mazić et al., (2014) found a high correlation between DEXA and BMI as indicators of adiposity in athletes.

- Plethysmography: In this test, body volume is measured by air rather than water displacement. One commercial system uses a dual-chamber plethysmograph that measures body volume by changes in pressure in a closed chamber. It has been shown as a valid and reliable method in the general population (ACSM, 2018).

- Bioelectrical impedance analysis (BIA): the accuracy of BIA is similar to skinfolds, as long as a stringent protocol is followed and the equations programmed into the analyzer are valid and accurate for the population being tested (ACSM, 2018). Rieken et al. (2011) reported that BIA is more accurate at assessing nutritional status than the measurement of skinfold thickness.

- Near-infrared intercadence requires additional research to substantiate the validity and accuracy for body composition assessment.

3. b) Indirect field tests for the study of body composition:

It is based on anthropometric measures that include height, weight, waist and hip circumferences, and skinfolds. Although skinfold measurement is more difficult than other anthropometric procedures, it provides a better estimation of body fat than those based only on height, weight, and circumferences (ACSM, 2018).

The BMI shows the degree of overweight and obesity, is used to assess weight related to height and is calculated by dividing weight in kilograms by height in m^{-2}. These are simple measurements that provide a convenient and inexpensive alternative for estimating body composition, and thus are frequently used in clinical studies and fitness conditioning.

- Skinfold Measurements: body composition determined from skinfold measurements correlates well (r = .70 to .90) with body composition determined by hydrodensitometry.

The principle behind this technique is that the amount of subcutaneous fat is proportional to the total amount of body fat. It is assumed that close to one third of total fat is located subcutaneously. The exact proportion of subcutaneous-to-total fat varies with sex, age, and ethnicity. Therefore, regression equations used to convert sum of skinfolds to BF% must consider these variables for greatest accuracy (ACSM, 2018).

3.13 Rate of Perceived Effort (RPE)

In studies on physical work it is important to assess various subjective symptoms, complaints, and annoyances. To measure such symptoms, psychophysical ratio scales may be used, along with simpler category rating scales. These types of ratio scaling methods are very useful when one wants to describe how the subjective intensity varies with the physical intensity. The category scale, commonly referred to as the Borg Scale (Borg et al., 1982), has been widely used to study the rate of perceived effort (RPE) during exercise in laboratory, occupational and clinical settings. This scale was developed by Borg so that the perceptual ratings increased linearly with power output and heart rate on a cycle ergometer (G. Borg, 1987).

Then, Borg et al. (1990) developed a new psychophysical scale with perceptual ratings that increase as a positively accelerating function. The score was based on the Borg category ratio scale and consisted of a scale ranging from 6 to 20 (Table 9).

Table 9. There are many adapted scales for different ages and sport, all of them based in the original one (Borg, 1982)

6	*No exertion at all*
7	*Extremely light*
8	*Very light*
9	
10	*Light*
11	
12	
13	*Somewhat hard*
14	*Hard*
15	
16	
17	*Very hard*
18	*Extremely hard*
19	
20	*Maximal exertion*

3.14 Energy Metabolism during Exercise

Phosphocreatine (PCr) breakdown through the creatine kinase reaction and glycogen breakdown into lactate (glycogenolysis/glycolysis) are the primary pathways of ATP provision during short exercise events requiring maximal force and power output (PO). For example, during a 6-second sprint, during which PO corresponds to ~250% of VO_{2max}, PCr hydrolysis and glycogenolysis each contribute ~50% of the total ATP (adenosine triphosphate) requirement with very little contribution from oxidative phosphorylation (Saris et al., 2003). The relative contribution of PCr to energy turnover increases as the exercise duration becomes shorter. Conversely, during maximal exercise lasting 5 to 6s to about 90s to 2min, glycogen degradation to lactate rather than the creatine kinase reaction is primarily involved in the regeneration of ATP (Mujika, 2012).

ATP turnover rate under normal conditions is not a limiting factor in short 'explosive' exercise performance (throwing, jumping, weight lifting etc.). Muscle structure (physiological cross sectional area (CSA), muscle length, muscle architecture), neural factors (neuromotor drive, coordination) as well as skill ability are the predominant factors limiting performance. However, when sprint exercises cause PCr depletion and involve substantial activation of glycogenolysis, the rate of ATP breakdown often exceeds the rate of ATP synthesis, which results in net ATP breakdown and increasing intracellular content of the end products of adenine nucleotide degradation, notably inorganic phosphate (Pi), adenosine diphosphate (ADP), adenosine monophosphate (AMP), inosine phosphate (IMP) and ammonia. A small proportion of the IMP formed is converted to inosine and further to hypoxanthine, which appear in the venous effluent of muscles (Saris et al., 2003). There is substantial evidence to indicate that hydrogen ion (H+) production (~pH drop) from glycolysis/glycogenolysis in excess of available buffer capacity, in conjunction with intracellular accumulation of ADP and Pi resulting from adenine nucleotide degradation, are implicated in fatigue during short maximal exercise (Mujika, 2012).

3.14.1 Energy Expenditure (EE)

There are several ways to measure or estimate energy expenditure (EE). Methods for measuring EE range from direct but complex measurements of heat production (direct calorimetry), to relatively simple indirect metabolic measurements (indirect calorimetry), and from very expensive tracer methods (doubly labelled water) to relatively inexpensive and convenient estimations of EE (heart rate monitoring and accelerometry).

3.14.2 Substrates Oxidation Rates (FATox and CHOox)

During prolonged exercise, CHO and FAT are the primary substrates oxidized to fuel energy metabolism (Romijn et al., 1993; van Loon et al., 2001). Humans predominantly store carbohydrates as glycogen in skeletal muscle (Bergström et al., 1967) and the liver (Nilsson et al., 1973) with modest quantities also found in the brain, kidneys, and adipose tissue (Meyer et al., 2009), and ∼4 g circulating in plasma as glucose (Wasserman, 2009).

Carbohydrate and fatty acids are the main fuels oxidized by skeletal muscle to provide energy during aerobic exercise at intensities between 30 to 80% VO_{2max} (Van Loon et al., 1999). However, at a given exercise intensity and metabolic demand, there can be reciprocal shifts in the proportions of CHO and FAT that are oxidized (Spriet, 2014).

It has been shown that exercise intensity is one of the main factors that influences substrate utilization during exercise. Shifts in energy substrate mobilization and utilization occur as exercise intensity increases (Achten, 2002). There is a progressive increase in the relative contribution of CHOox to EE and a corresponding decrease in the relative contribution of FATox to EE. However, from low to moderate intensities of exercise, the absolute rate of FATox increases and then declines as exercise becomes even more intense (Horton et al., 1998; Randell et al., 2017; San-Millán & Brooks, 2017; Spriet, 2014). Several mechanisms have been proposed to explain the lower FATox rates at high compared with moderate exercise intensities. It has been shown in

athletes that after endurance training, FATox at a given intensity is increased and coincides with increases in performance (Jeukendrup, 2011; San-Millán & Brooks, 2017). These observations indicate that the ability to oxidize fatty acids is related to improved performance. These changes are likely to be the results of an overall increase in aerobic capacity (Randell et al., 2017).

Therefore, it is necessary to use an exercise protocol to determine substrate oxidation rate (FATox and CHOox), such as a continuous incremental exercise test on a cycle (Achten, 2002) or treadmill (Mohebbi & Azizi, 2011) with 3-min stages and increments of 35-W and 1km/h respectively, at a work rate that allows for valid assessment of Fat_{max} and a Fat_{max}zone in well-trained athletes.

Fat Oxidation

Lipids are the major fuel (approximately 60%) for non-contracting skeletal muscles and the body at rest (Brooks, 1997). Lipids stores are sufficient to ensure physical activity for several hours, or even several days, even in the thinnest athletes (Mujika, 2016). Indeed, given that 1g of fat provides ~9.75 kcal of energy (Jeukendrup, et al., 1998), it can be estimated that even very lean individuals of 70 kg and 10% body fat possess ~68,250 kcal (7,000 g) of endogenous fat energy.

Human fat energy storage is effectively unlimited in the context of exercise (Gonzalez et al., 2016), and so identifying the determinants of, and enhancing, FATox during exercise is a pertinent training and research goal in endurance sports. Indeed, FATox capacity has been correlated with performance in Ironman triathlons, which are ultra-endurance events (>8 h) in which CHO availability is likely limiting (Frandsen et al., 2017). Studies demonstrated that increased lipid availability in the form of plasma free fatty acids (FFAs) increased FATox and decreased CHOox in muscles (Spriet, 2014).

Lastly, fat metabolism is of great relevance in a health setting, given the observed positive and negative relationships between 24-h FATox and markers of metabolic health such as insulin sensitivity and weight gain (Robinson et al., 2015), and that the

capacity for FATox during exercise has been associated with insulin sensitivity, metabolic flexibility (San-Millán & Brooks, 2017), and lower metabolic risk factors (Achten et al., 2002; Robinson et al., 2015).

Fat oxidation increases from low to moderate exercise intensities and decreases from moderate to high exercise intensities. Perhaps the most fundamental determinant of whole-body FATox rate is exercise intensity. The relationship between exercise intensity and FATox is generally parabolic; with FATox initially increasing with exercise intensity before declining at high work rates (Romijn et al., 1993).

Generally, the highest rates of FATox are found at low to moderate exercise intensities (range 33-65% VO_{2max}) (Achten & Glesson, 2002; Achten, 2003; Randell et al., 2017; San-Millán & Brooks, 2017; Saris et al., 2003). Most studies, however, measured FATox at only two (Randell et al., 2017; Spriet, 2014), three (van Loon et al., 2001), or four (Achten et al., 2003) different exercise intensities. This makes it difficult to accurately determine the exercise intensity that elicits maximal FATox. To our knowledge, there are no papers in the literature that have systematically studied FATox over a large range of exercise intensities to identify the exercise intensity at which FATox is maximal. Previous studies demonstrated that training status had a significant effect on substrate utilization (Van Loon et al., 1999).

Fat oxidation during exercise is more difficult to measure in non-active individuals compared to athletes because the rates of FATox are lower relative to the measurement error. For an overview of methods for measuring fat metabolism see Table 10.

Table 10. Fat oxidation

Method	Reproducibility (CV in %)	Precision	Advantages	Disadvantages	References
Respiratory exchange ratio	~3–5%	Good	-Metabolic measurements possible -Exaggerates differences	-Difficult to measure in the field -Does not give information about the source of fatty acids oxidised	(Carter & Jeukendrup, 2002)
Stable isotopes (13C-palmitate infusion)	?	Good	Gives an indication of the source of the fatty acids oxidised Useful in combination with indirect calorimetry Relatively non-invasive	-Can only be used in steady-state conditions -Can only be measured in lab conditions -Is a whole body measurement and not specifically muscle -Specialised equipment and personnel needed to perform analysis	(Wolfe, 1992)
Muscle biopsy	26%	Good	Direct measurement of intramuscular triglyceride concentration	-Invasive -Large variability depending on the site of the biopsy -Samples easily contaminated with adipose tissue	(Wendling, Peters, Heigenhauser, & Spriet, 1996)
^{13}C-NMR	6%	2012	-Direct measurement of intramuscular triglyceride concentration -Can distinguish between intra- and extramyocellular triglycerides -Non-invasive	-Potential problems with localisation -Expensive -Can only be performed in specialised hospital units	(Jeukendrupet al., 1998)

The "Fat_{max}" Test

The concept of Fat_{max} (maximal fat oxidation) has received a great deal of attention in recent years (Jeukendrup & Achten 2001; Achten et al., 2002; Achten, et al., 2003). This is due to an effort to recognize that facilitating fat metabolism is of importance for both aerobic performance and health-related benefits.

The specific intensity at which the FATox rate is maximal (commonly presented as a percentage of VO_{2max}) is defined as Lipoxmax, Fatoxmax, or Fat_{max} by different researchers (Brun et al., 2011) and provides a measurement of MFO: the highest rate of FATox observed at various intensities (Randell et al., 2017).

Fat_{max} was defined as the intensity where the greatest FATox was observed. In order to comprehensively define the relationship between whole-body FATox rates and exercise intensity, the "Fat_{max}" test was developed (Achten, et al., 2002). This protocol describes FATox over a wide range of exercise intensities, is relatively quick and allows measurements to be recorded in a single visit to the laboratory. This graded exercise test elucidates whole-body FATox rates across a range of exercise intensities, the maximal rate of FATox (MFO), and the intensity at which the MFO occurs (Fat_{max}) using indirect calorimetry (Figure 9). The protocol, often called a FAT_{max} test, provides a measure of maximal FATox, as well as the exercise intensity (most commonly represented as a % VO_{2max}) at which MFO occurred (FAT_{max}). First developed on a cycle ergometer, the test involves continuous increases in work rate, every 3 min by 35 W, until exhaustion. Throughout the test, breath-by-breath measurements are obtained and rates of FATox are calculated (using stoichiometric equations) for each stage of the test. Thanks to this inaugural study, a treadmill Fat_{max} test protocol has also been developed (Achten, et al., 2003). In addition, studies investigating the reproducibility of MFO and FAT_{max}using this test protocol have found small intraindividual variation (Achten, 2003).

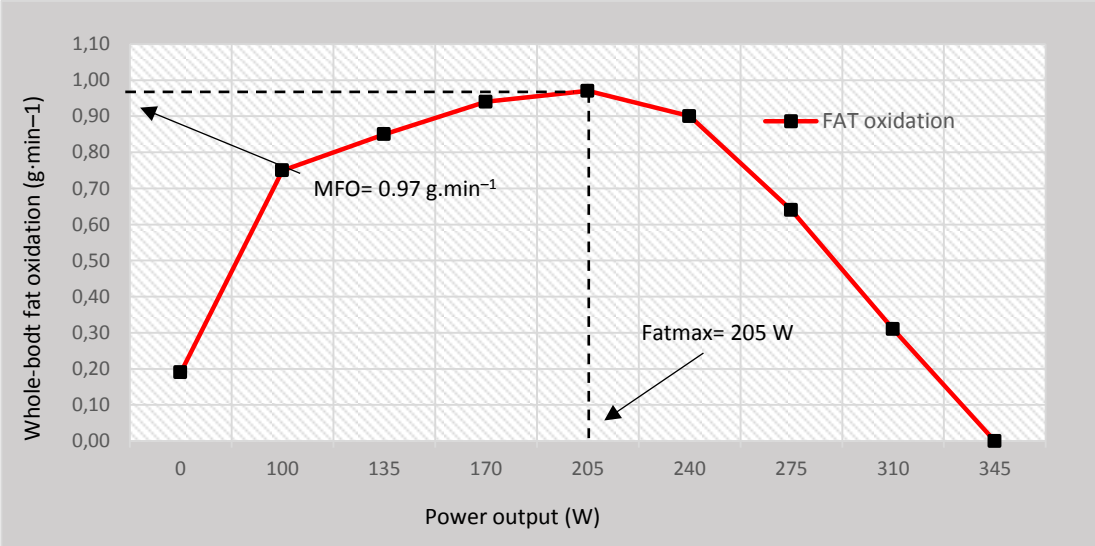

Figure 9: Representative illustration of fat oxidation (g.min–1) against exercise intensity (W) during a graded, cycling Fatmax test.

Carbohydrate Oxidation (CHO)

Human CHO storage is finite, and typically amounts to <3,000 kcal (<740 g) (Gonzalez, et al., 2016), ~80% of which is in skeletal muscle and ~10–15% in the liver (Jensen et al., 2011). The main CHO sources are muscle and liver glycogen, liver gluconeogenesis, and ingested CHOs (Van Loon et al., 1999). The relative contribution of these fuel sources also varies with exercise intensity (Brooks, 1997; Coggan, 1997) and training status (Van Loon et al., 1999).

Carbohydrates are the quantitatively most important metabolic substrate during prolonged exercise at moderate-to-high intensities (Romijn et al., 1993; Van Loon et al., 1999), and skeletal muscle glycogen can become depleted to near-zero concentrations after exercise of sufficient length and intensity (Bergström, et al., 1967; Bergström & Hultman, 1967). The availability of CHO reserves as a substrate for muscle metabolism, and for the central nervous system, constitutes a key factor in performance during activities of extended duration (Mujika, 2012).

Since endogenous CHO stores are limited, depletion of these stores may occur within several hours of exercise. Depletion of carbohydrate stores (liver glycogen and/or muscle glycogen) have been recognised as causes of fatigue. Prevention of glycogen depletion can enhance performance and it is generally believed that an increased delivery of substrate from exogenous sources is beneficial. During exercise, breath 13CO2 will become enriched and together with a measurement of the total CO_2 production rate, exogenous CHOox rates can be quantified.

Muscle Glycogen

Muscle glycogen and blood glucose are the most important substrates for contracting muscles (Romijn et al., 1993). Muscle glycogen is an important substrate for muscular work. When exercise intensity is 65–90% it is usually the most important substrate. Glycogen stores can potentially be exhausted when the duration of exercise exceeds 90 mins (Mujika, 2016).

Fatigue during prolonged exercise is often associated with muscle glycogen depletion and reduced blood glucose concentrations (Asker & Jeukendrup, 2011) and, therefore, high pre-exercise muscle and liver glycogen concentrations are believed to be essential for optimal performance, although it is unlikely that any of these factors alone limits prolonged exercise performance. Studies in the 1960s demonstrated that muscle glycogen depletion is one of the main causes of fatigue (Bergström & Hultman, 1967). Since then, there has been enormous interest by exercise physiologists in studying the role of muscle glycogen, and a variety of techniques have been used to measure muscle glycogen concentration, muscle glycogen breakdown, or both.

Muscle glycogen is also thought to be important for recovery and many studies have investigated the effects of nutritional and performance interventions on the rate of muscle glycogen synthesis after depletion. When carbon isotope (^{13}C) labelled glucose is infused at a constant rate of disappearance (Rd), glucose can be determined. In most conditions the Rd glucose will be equal or very similar to the rate of plasma glucose oxidation (Jeukendrup et al., 1999).

Measuring FAT and CHO Oxidation – Gas Exchange

Fat and CHO oxidation rates can be measured using indirect calorimetry. Gas exchange measurements not only allow for an estimation of EE but also of the substrate mixture used. Krogh and Lindhard (1920) in the beginning of the 20th Century used the inherent differences in chemical properties of CHO, fat and protein to obtain information about fuel utilisation. The respiratory quotient reflects the relative contribution of CHO and fat to total EE. Absolute rates of FATox can then be calculated using stoichiometric equations (Frayn, 1983).

The application of the respiratory exchange ratio (RER) is based on the premise that the exchange of O_2 and CO_2 at the mouth represents the processes that occur in the tissues that oxidise the fuels. This assumption is valid at rest and during exercise up to about 80–85% VO_{2max}, above which RQ measured at the mouth does not always reflect the oxidation processes in cells (due to hyperventilation and excess CO_2 output). Normally FATox increases from low to moderate intensities, peaks around 64% VO_{2max}

in trained individuals and becomes negligible around 80% VO_{2max} (Achten et al., 2003). In order to demonstrate the effect of an intervention on whole body FATox, it is advisable to measure FATox over a wide range of exercise intensities as discussed in a recent paper by Achten et al., (2003).

Gas exchange measurements will only allow conclusions to be drawn about total whole body FATox, but the source of FFAs cannot be identified. Whether the fatty acids oxidised are derived from adipose tissue, or triglycerides (IMTG) can only be determined with the use of isotopic tracers or magnetic resonance spectroscopy or imaging techniques (Gonzalez et al., 2016).

Reductions in FATox have been observed after CHO ingesting, with increasing CHO content in the diet. Other factors like exercise intensity and exercise duration also affect FATox rates. Training not only lowers FATox but also changes the source of the fatty acids oxidised. The importance of increased FATox rates is subject to current debate. A claim has been made that increased FATox during exercise reduces the breakdown of CHO, though the relevance is unclear unless it can be demonstrated that glycogen depletion can be prevented (Saris et al., 2003).

The coefficient of variation of rates of FATox, measured by RER at a given exercise intensity are between 10 and 30%, whereas the coefficient of variation (CV) of the intensity at which maximal FATox occurs has a coefficient of variation between 6-10% (Achten et al., 2003). In order to minimise variation in the measurements and decrease the coefficient or variation of all fat metabolism related parameters.

Indirect Calorimetry

Indirect calorimetry is one of the most common tools in exercise physiology, and provides one the most sensitive, accurate, and noninvasive measurements of EE in an individual. It is used for various purposes, including the assessment of aerobic power, determination of exercise intensity and the measurement of EE (Carter & Jeukendrup, 2002). This method remains a gold standard in measuring EE in research and clinical settings (Haugen, Chan, & Li, 2007).

Indirect calorimetry is the method by which the type and rate of substrate utilization and energy metabolism are estimated in vivo starting from gas exchange measurements (CO_2 production and O_2 consumption during rest and steady-state exercise). An RER of 0.7 indicates that fat is the predominant fuel source, a value of 1.0 is indicative of CHO being the predominant fuel source, and a value between 0.7 and 1.0 suggests a mix of both fat and CHO. As exercise intensity increases and CHO become the dominant or primary fuel, the respiratory quotient and the RER increase to between 0.9 and 1.0. The RER can also exceed 1.0 during intense exercise.

Therefore, using a short-duration step protocol and continuous indirect calorimetry, whole rates of FATox and CHOox can be estimated across a range of exercise workloads. This method is of interest in endurance sports. Studies show that endurance training increases FATox during progressive maximal exercise.

3.14.3 Blood Lactate Concentration

Blood lactate is another measurement that is often associated with quantifying metabolic stress associated with exercise. The traditional approach to using [La^-] as a marker of exercise intensity is to develop a [La^-] concentration/workload relationship, or simply to measure the concentration of blood lactate after a bout of exercise. Blood lactate concentration during exercise is often used as a marker of exercise intensity and training status (Swart & Jennings, 2004).

Lactate is a product of oxygen-independent metabolism of glycogen via the glycolytic pathway. Lactic acid, which appears in the muscle and blood during exercise, is a product of pyruvate/lactate conversion in the process of glycolysis, which regulates cytosolic NADH+H/NAD equilibrium thus making possible anaerobic generation of ATP. For each molecule of glucose metabolised, two molecules of either lactate or pyruvate are formed. [La^-] levels are a result of the balance between lactic acid production in muscle and elimination from the bloodstream, the main pathways of [La^-] elimination being 1) uptake in the liver for oxidation and gluconeogenesis and 2) uptake in active as well as inactive muscles for oxidation. Lactate levels in arterial blood are considered to be a good reflection of the whole body status of lactate production and

elimination. While low levels of exercise are not associated with arterial lactate increase, high exercise intensities induce a rise in [La^-] and metabolic acidosis. Thus, during low-intensity exercise, muscle lactate production is in balance with lactate elimination, while this balance is disturbed during higher exercise intensities during which lactate production is enhanced more than lactate elimination (Billat, 1996; Swart & Jennings, 2004). The increase of lactate production during high intensity exercise is associated with a need of additional energy formation over what can be provided by aerobic pathways. In these conditions, conversion of pyruvate to lactate becomes increasingly important for an adequate regeneration of ATP. The metabolic acidosis resulting from the increase of lactic acid is one of the mechanisms of muscle fatigue, limiting the duration of high intensity exercise. Between the two states of [La^-] balance there is a transition area of intensities of exercise in which the shift to lactate accumulation occurs (Table 7). This area can be identified as "lactate threshold", "onset of blood lactate accumulation", "maximal steady state of blood lactate level" or, the most frequent but perhaps the most imprecise, "anaerobic threshold" (Billat, 1996)

According to the '*anaerobic threshold'* theory, the change from oxygen-dependent to oxygen-independent metabolism is presumed to be a result of a limit in the supply of oxygen to working muscles at higher exercise intensities. Increases in cardiac output are insufficient to meet the demands for oxygen in working muscles and the production of lactate from oxygen- independent metabolism is presumed to then cause metabolic acidosis (Messonnier et al., 2013).

Recents studies show that the presence of lactatemia leads to impaired FFAs clearance and elevated plasma FFAs concentration, another symptom of some metabolic diseases such as insulin resistance. Blood lactate accumulation is negatively correlated with FATox and positively correlated with CHOox during exercise across populations with widely ranging metabolic capabilities (San-Millán & Brooks, 2017).

Lactate / Ventilatory Threshold

The exercise intensity corresponding to the increase in [La^-] above resting levels (lactate threshold (LT)) and the associated changes in gas exchange (ventilatory threshold (VT)) are powerful predictors of endurance performance (Jones & Carter, 2000; Jones AM, 1988; Messonnier et al., 2013).

The lactate threshold is defined by the lowest intensity at which lactate production exceeds the muscle's oxygenation capacity. A significant increase in muscular oxidation capacity is one of the main factors explaining the high lactate threshold values observed in top-level endurance athletes. A possible approach for evaluation of the status of [La^-] accumulation is to determine the area of exercise intensities which represent the transition between the intensities corresponding to the steady state of [La^-] and those which induce [La^-] increase. According to this traditional theory, the exercise intensity coinciding with the '*lactate threshold*' is well defined and is an accurate marker of training status. It has been suggested that training at and above the 'lactate threshold' will result in adaptations that reduce the concentration of blood lactate during subsequent submaximal and maximal exercise. A common assumption is that training at the intensity coinciding with the LT will cause an improvement in lactate clearance or a decrease in lactate production at submaximal workloads. This theory has promoted the testing of blood lactate concentration in the field as a marker of training intensity and training status. This is based on the assumption that [La^-] concentrations are repeatable at controlled workloads and that the concentration of [La^-] decreases as training status improves (Swart & Jennings, 2004).

Lactate is a key element of performance and well-trained athletes have a higher lactate clearance capacity and decreased [La-] levels at the same relative and absolute submaximal exercise intensities, owing to mitochondrial abundance and function (San-Millán & Brooks, 2017). However, [La^-] accumulation in response to high intensity exercise can be modified by training status (the more highly trained subject having lower lactate levels at a given intensity of exercise), by modifications of oxygen supply to the exercising subject (hypo- or hyper-oxia) and/or by administration of exogenous nutritional or other products. The claims of such products to modify lactate

accumulation should be substantiated by methods enabling the detection of lactate accumulation and, eventually, associated metabolic acidosis.

Exercise-induced lactic acidosis results in the consumption of HCO_3-ions for buffering hydrogen ions and, consequently, in the rise of CO_2 production. When measuring VCO_2 as a function of VO_2, then the exercise intensity at which the VCO_2 increase is accelerated corresponds to the onset of metabolic acidosis and, thus, of [La^-] accumulation (designated as "ventilatory threshold") (Saris et al., 2003). Ventilatory expired gas responses are often used in fitness tests as an estimation of the point at which lactate accumulation in the blood occurs. Assessment of this physiologic phenomenon through ventilatory expired gas is typically referred to as VT. Several different methods using ventilatory expired gas responses exist for the estimation of this point. These include the ventilatory equivalents and V-slope method (López Chicharro, J., & Izquierdo Redín, 2006). Whatever approach is used, it should be remembered VT provides only an estimation, and the concept of anaerobic threshold during exercise is controversial (ACSM, 2014). Because exercise beyond the LT is associated with metabolic acidosis, hyperventilation, and a reduced capacity to perform work, its estimation is a useful physiological measurement when evaluating interventions in patients with heart and pulmonary disease as well as studying the limits of performance in apparently healthy individuals. However, it should be noted that secondary to abnormal ventilatory responses observed in a significant proportion of patients with CHF (i.e., exercise oscillatory ventilation), determination of VT may not be possible (ACSM, 2018). In addition to estimating when [La^-] values begin to increase, maximal minute ventilation (VE_{max}) can be used in conjunction with the maximal voluntary ventilation (MVV) to assist in determining if there is a ventilatory limitation to maximal exercise.

Numerous studies also testify to the sensitivity of LT and VT to endurance training (Jones & Carter, 2000; Messonnier et al., 2013; San-Millán & Brooks, 2017) (Figure 10). A rightward shift of the LT/VT to a higher power output or running speed is characteristic of successful ET programmes.

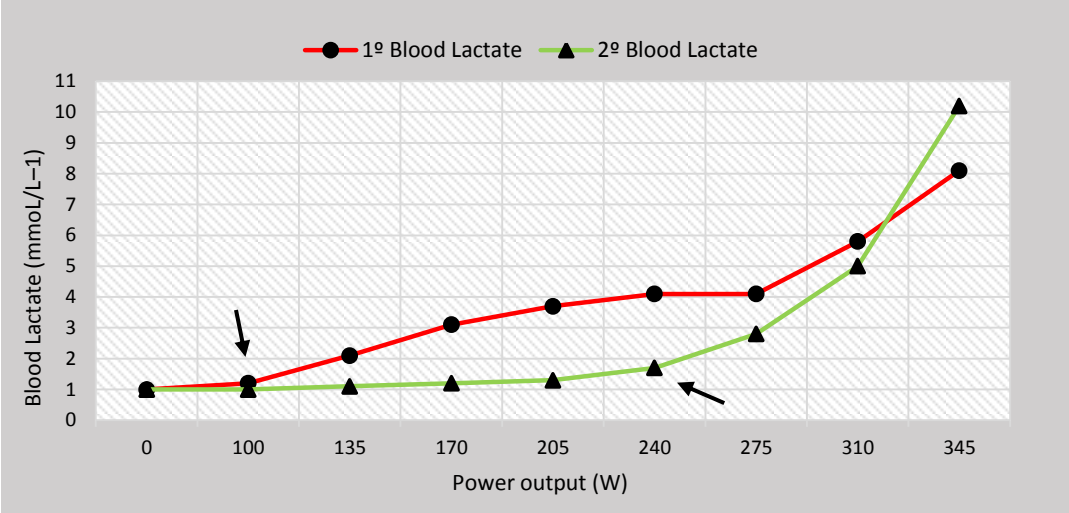

Figure 10: The effect of 6 weeks of ET on [La-] response to incremental exercise in a typical individual. The vertical arrows denote the LT determined before (1º) and after (2º) training.

Measurement of Blood Lactate Accumulation

The recent development of portable blood lactate analyzers has made it relatively easy to test blood lactate concentration in the field. The most simple and most widely used method of [La^-] monitoring is the measurement of lactate concentration in blood samples. Four sites are commonly used for blood sampling: forearm arteries, forearm veins, earlobes and fingertips, with the role of sampling size is mentioned below. The blood sample is analysed mostly by enzymatic methods for [La^-] concentrations (Billat, 1996).

[La^-] accumulation during exercise may be assessed by monitoring the evolution of [La^-] concentrations during exercise with increasing levels of intensities. Therefore, the lactate levels in arterial blood are considered to be a "gold standard" for evaluation of lactate balance as they do reflect the whole body status of [La^-] and elimination (Table 11).

Table 11. A classification of the different terminologies that exist in the literature to define specific changes in the exercise blood lactate response adapted from Billat (1996) and Saris et al., (2003)

Blood lactate value (mmol/L)	Definition and designation	Protocol
Baseline +1	Onset of plasma lactate accumulation: the VO_2 observed during the incremental exercise with a [La^-] concentration that is 1 mmol/L above the baseline [La^-] level	Discontinuous incremental test with 8 stages, of 10 min
2.2	Maximal steady-state: the oxygen, heart rate and/or treadmill velocity at which plasma lactate level was 2.2 mmol/L	2 discontinuous stages, of 10 or 15 min
2.5	Lactate threshold: the exercise intensity that elicits a [La^-] level of 2.5 mmol/L after 10 min of exercise	Discontinuous incremental test with stages of 10 min
4	Anaerobic threshold: the VO_2 or velocity associated with a [La^-] concentration of 4 mmol/L	Continuous incremental test with stages of 3 min
4	Onset of [La^-] accumulation	Continuous incremental test with stages of 4 min
2-7	Individual anaerobic threshold: metabolic rate where the increase of [La^-] is maximal and equal to the rate of diffusion of lactate from the exercising muscle	Continuous incremental test with stages of 4 min with measurement of the lactate time course after the test
3.5-5	Lactate threshold: the starting point of an accelerated lactate accumulation around 4 mmol/L and expressed in %"il02max	Continuous incremental test with stages of 3 min
2.2-6.8	Maximal steady-state of [La^-] level: the exercise intensity (W_{CL}) which produces the maximal steady-state of [La^-] level	2 submaximal intensities (60-65% and 75-80% of VO_{2max}) of 20 min carried out on the same day and separated by 40 min of complete rest

Abbreviations: VO_2 = oxygen uptake; VO_{2max} = maximal oxygen uptake.

3.14.4 Metabolic Flexibility

Human physiology needs to be well adapted to cope with major discontinuities in both the supply of and demand for energy. This adaptability requires 'a clear capacity to utilize lipid and CHO fuels and to transition between them' (Kelley et al., 2002). Such capacities characterize the healthy state and can be termed '*metabolic flexibility'* (Table 12). Metabolic flexibility and inflexibility are terms proposed well over a decade ago by Kelley and colleagues (Storlien et al., 2004), and are gaining popularity among researchers and clinicians working with CVD, T2DM and obesity. Metabolic flexibility reflects the ability

Table 12. Key points of Metabolic Flexibility adapted from San-Millán & Brooks, (2017)

Measurements of [La-] concentration and FATox provide an indirect method to assess metabolic flexibility and oxidative capacity during exercise in different populations.
The inverse correlations between [La-] and FATox are quite robust, therefore assessing [La-] alone could be an effective way to indirectly assess mitochondrial function and metabolic flexibility during exercise in different populations.
Since lactate exerts profound effects on fat and CHO metabolism, a poor mitochondrial lactate clearance capacity due to decreased mitochondrial function could greatly affect FATox and CHOox.

to oxidize fat and CHOs, which is needed to match fuel availability with metabolic responses and meet large increases in energy demands. Increased metabolic flexibility, a high capacity to oxidize lipids and a later transition from FATox/CHOox, as well as elevated [La-] as exercise PO increases, are characteristic of endurance athletes (San-Millán & Brooks, 2017, while decreased oxidative capacity and higher [La-] concentrations are characteristic of individuals with low aerobic fitness at the same absolute submaximal exercise intensities (Messonnier et al., 2013). San Millán & Brooks (2017) showed the presence of an overarching major effect of lactatemia on limiting FATox in individuals of widely ranging exercise capacities. In 1994, Brooks and Mercier proposed the 'crossover concept' (CO) (Brooks, 1997) as a novel approach to studying CHO and fat metabolism during exercise (Figure 11).

Cross-sectional (Messonnier et al., 2013) and longitudinal training studies (Bergman et al., 1999a) on healthy, young individuals show that training lowers circulating [La-] by increasing lactate clearance (Bergman et al., 1999a), increasing lipid oxidation (Bergman et al., 1999b), and decreasing glucose and total CHO utilization (Bergman et al., 1999c) during exercise at given absolute exercise PO. San-Millán & Brooks (2017) showed consistent and strong inverse correlations between [La-] and FATox, and since both lactate and fatty acids are mitochondrial substrates, measurements of [La-] and FATox rates during exercise provide an indirect method for assessing metabolic flexibility and oxidative capacity across individuals of widely different metabolic capabilities (San-Millán et al., 2009; San-Millán & Brooks, 2017).

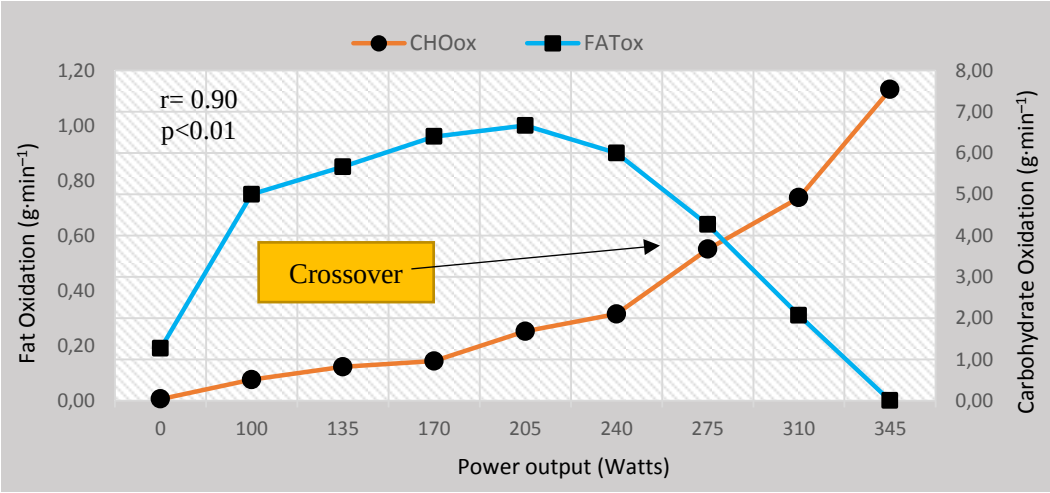

Figure 11: Representative illustration of 'crossover effect' (Brooks, 1997)

3.15 Thermoregulation during Physical Exercise

The human thermoregulatory system is complex, and the influence of exercise on the system is complicated. Exercise promotes several physiological and thermal changes, leading to changes in thermal homeostasis of the human body, metabolic rate (M) variation and increased internal heat.

During physical exercise, human thermoregulatory functions are critical for survival and sustenance of physical work. Thermoregulatory balance during strenuous exercise depends on the interaction of metabolic heat production and exchange with the environment. Studying heat generation and dissipation during strenuous PA is of great interest due to the significant increase in BT compared with the resting state, which may lead to hyperthermia and reduced efficiency. In these conditions, thermal regulation processes play a major role in maintaining BT within the narrow range tolerated by the body.

Humans have a remarkable ability to adapt and sustain physiological functions in different conditions of exercise. When heat production exceeds heat dissipation (physical activity or high Ta), BT increases, which activates the adaptation mechanisms of dissipation of excessive heat (Etain et al., 2015).

Physical exertion poses a challenge to thermoregulation by causing a substantial increase in metabolic heat production. During PE, metabolic heat production can increase 10- to 20-fold, but less than 30% of the heat generated is converted to mechanical energy (Périard et al., 2014). During exercise, the increase in metabolic heat production increases the rate at which heat must be dissipated to the environment to prevent dangerous elevations in tissue temperature. In the case of increased heat production, including intense PE, the activation of heat dissipation is carried out both by changes in the skin's vascular tone and increased perspiration. Increases in heat loss via cutaneous vasodilation and sweating induced by the activation of the autonomic nervous system facilitate increases in dry heat exchange (primarily convection and radiation) and evaporative heat loss, respectively. Evaporative heat loss takes place when sweat changes from liquid to gaseous states. During PE, >80% of heat is dissipated through

evaporative heat loss, making it the primary means of heat removal from the body (Gisolfi, 1993). Therefore, the ability to sweat is very important for thermoregulation and the sustenance of exercise over long periods. About 1 litre of sweat is lost for each hour of exercise in hot conditions, and higher sweat rates (>2 L/h) have been reported in well-trained athletes (Gisolfi, 1996). However, the propensity for sweat to be evaporated is inversely related to the amount of water vapour in the air. A high relative humidity inhibits evaporative heat loss whereas evaporative heat loss is promoted when relative humidity in the air is low. Exercising in a warm and humid environment causes the body to lose fluid through sweat loss with minimal heat loss.

For competitive athletes and active individuals, the effective dispersal of the heat load generated by contracting muscle bears particular importance. Failure of mechanisms to effectively remove body heat during strenuous exercise could result in substantial decreases in physical performance (Rowland, 2008). During exercise, the body stores heat as a result of an imbalance between the rate of heat gain and heat loss. Under compensable conditions, heat balance is achieved within 30–45 min of steady-state exercise, with the greatest rate of body heat storage, and therefore increase in Tc, occurring in the first 15–20 min (Kenny & Jay, 2013). During the performance of intermittent exercise, a greater increase in evaporative heat loss occurs following initial exercise, such that the amount of heat stored in successive exercise bouts is reduced by as much as 40–60% under compensable conditions (Kenny et al., 2009; Kenny & McGinn, 2016). This has been associated with either a priming effect induced by the progressive increase in body Tc (Kenny et al., 2009; Kenny & McGinn, 2016; Taylor et al., 2014) and/or enhanced peripheral and/or central adaptations of thermo-effector activity (Gagnon & Kenny, 2011). However, as discussed below, although these conditions may alter the body's capacity to dissipate heat during exercise-induced heat stress, differences may only be evidenced above a given heat load threshold (i.e., exercise intensity) (Kenny & McGinn, 2016; Stapleton et al., 2014).

In summary, then, thermoregulatory efficacy during exercise is most closely linked to:

1. Adequacy of circulatory responses
2. Rate of sweat production

3. Maintenance of body fluid volume,

all in response to exercise intensity (Gant et al., 2004; Rowland, 2008).

There are numerous factors which can enhance or limit the capacity for heat loss during exercise, including ambient conditions and clothing, hydration status, advanced age, reduced VO_{2max}, energy substrate utilization, body composition, wind velocity, the presence of chronic diseases, and others (Kenny & Jay, 2013). Also some biological determinants of exercise thermoregulation, including body mass, surface area-to-mass ratio, and sweat rate, are gender-discrete variables with the potential to alter the exercise-thermoregulatory response to different environments, fluid intake, and exercise metabolism. There have been extensive studies examining the influence of these factors on thermoregulatory function during exercise.

Numerous individual and external factors can influence BT, but consensus is lacking on which factor, or set of factors, has the most impact during prolonged physical exertion during endurance events and race-like circumstances. Environmental conditions, dehydration, and metabolic rate are commonly referenced as limiting thermoregulatory control; gender may also contribute independently or in conjunction with other factors. However, some reports have shown that, when age, thermal acclimation, body size, maximal aerobic capacity, cardiovascular responses during exercise and relative workload are matched, thermoregulatory sex differences are relatively negligible (Nagashima et al., 2012).

3.15.1 Role of the Environment

In most places where we exercise, a variety of ambient temperatures are available and external insulation is easily adjusted. The thermodynamic properties of the environment can significantly add to the challenge that work imposes on human thermoregulation. Lind (1963) was the first to propose a ‘prescriptive zone’ of environmental temperatures dependent on exercise intensity for the prediction of a thermal steady state. As metabolic heat production rises with the intensity of exercise, the need to transfer heat away from the body becomes paramount for the maintenance

of thermal equilibrium. Environmental extremes can severely limit a favourable thermal gradient for heat loss, thus imposing additional thermoregulatory strain.

Performance in Hot Environments

Degraded physical performance and exhaustion can be caused by multiple physiological factors. Investigators studying the performance-limiting factors during exercise-heat stress have historically focused on the implications of the profound cardiovascular demand of simultaneously perfusing both exercising muscle and skin (González-Alonso, 2012).

Athletes frequently train and compete in temperatures of 25–30ºC (Cheuvront & Haymes, 2001). The avoidance of a large increase in Tc during exercise in such an environment is essential, given that the onset of fatigue has been found to be closely associated with the attainment of a 'critical' Tc (González-Alonso, 2012;González-Alonso et al., 1999a). The concept of premature fatigue in warm or hot environments as a consequence of elevated Tc was firmly established after several studies (González-Alonso et al., 1999a; Wong et al., 2016) demonstrated that an excessive rise in BT impairs endurance performance. It is well known that the limit of core body temperature is about 40°C in order to maintain prolonged exercise. Extremes in Tc (>42°C) can be detrimental to cellular and organ functions, which can threaten survival of the host (Lim et al., 2008). The central nervous system may also rely on hyperthermia to protect the body from "overheating". Hyperthermia may serve as a self-limiting signal that triggers central inhibition of exercise performance when a temperature threshold is achieved (Nybo, 2008).

When exercise is performed in the heat, the additional heat gained from the environment must be compensated through an increase in the rate of heat dissipation. During heat stress, skin blood flow increases, resulting in an increase in Tsk and an increase in heat dissipation to the environment. However, sustained exercise places high demands on body thermoregulatory mechanisms, especially in conditions of high Ta and humidity. In addition, high humidity suppresses evaporation of sweat on the skin (Etain et al., 2015).

Performance in Cool Environments

Instead, during mild thermal challenges, more subtle thermoregulatory mechanisms such as skin blood flow and NST are the key players. Contrary to heat stress, cold stress reduces blood flow to the skin, which leads to a decrease in Tsk and conservation of heat in the body. The ectothermic properties of Tsk and the endothermic properties of Tc function in synchrony to maintain thermal balance within the body. The central nervous system may also rely on hypothermia to protect the body from "overcooling". Hypothermia (Tc <35°C) impairs cardiovascular, respiratory and central nervous system functions, which can lead to muscle damage, pulmonary edema, hypotension, bradycardia, and renal failure (Lim et al., 2008).

Role of Acclimatization

Exercising in the heat represents a circulatory challenge for both unacclimated and acclimated athletes. Human thermoregulation and acclimatization are core components of the human coping mechanism for withstanding variations in environmental heat exposure. Human survival require increasing reliance on these mechanisms.

Heat acclimation provides an important thermoregulatory advantage, and the effect of heat acclimation on submaximal exercise performance can be quite dramatic, such that acclimated individuals can easily complete tasks in the heat that earlier were difficult or impossible. Heat acclimation may result in protective cellular adaptations. The intracellular heat shock protein (HSP) 72 is likely involved in maintaining cellular protein conformation and homeostasis during hyperthermia, inflammation, and injury, and a ten-day heat- and exercise-acclimation increased HSP72 in peripheral blood mononuclear cells. Exercise in humid heat appears to decrease the resting Tc in acclimated individuals (Patterson et al., 2004). Increases in both Tc and Tsk contribute to the various changes involved in heat acclimation (Nielsen et al., 1993).

Accordingly, heat acclimation/acclimatization mediates improved submaximal exercise performance by reducing physiological strain and abating a variety of other potential fatigue mechanisms (Nybo, 2008). Heat acclimation enhances heat dissipation

(i.e., through increases in SkBF and sweating), reduces Tc and Tsk, and enhances fluid balance and cardiovascular control during exercise in the heat (Lorenzo & Minson, 2010; Nielsen et al., 1993). Therefore, the time to exhaustion in heavy exercise will be affected by initial BT (Nagashima et al., 2012). There is evidence that individuals living and training many weeks in the heat might tolerate higher maximal Tc than those heat acclimated over 1 or 2 weeks (Sawka et al., 2012), and that trained individuals can tolerate higher Tc (González-Alonso et al., 1999b; Mallette et al., 2016; Périard et al., 2014).

3.15.2 Role of Age on Body Temperature

Age related reductions in whole body and/or local heat loss and/or increased body heat storage during exercise in the heat have been reported in a number of studies (Ho et al., 1997; Kenney et al., 1997; Larose et al., 2013; Stapleton et al., 2014; Tankersley et al., 1991).

Infants, children, and adults maintain comparable Tc. Heat loss is proportional to surface area and heat production is proportional to volume, so infants, who have a high surface to volume ratio, are at a higher risk of heat loss and hypothermia than adults. Young and older adults are particularly vulnerable to thermal extremes. Aging is a key modulator of the body's physiological capacity to dissipate heat, with adults as young as 40 yrs of age demonstrating marked impairments in heat loss (Larose et al., 2013). The elderly are also at risk of hypothermia; their thermoregulatory mechanisms are less efficient and lean body mass, which produces heat, diminishes with age (Campbell, 2011).

These impairments in heat loss are evidenced even in the very early stages of exercise (i.e., the first 10 min) (Kenny & McGinn, 2016; Larose, Wright, et al., 2013). In terms of the restoration of Tc in post-exercise, these age-related differences have recently been shown to further extend the time required to reestablish normal Tc. However, this is due solely to the greater amount of heat stored during exercise and not a more rapid and/or pronounced suppression of heat loss in the recovery period (Kenny & McGinn, 2016; Larose, Wright, et al., 2013). For example, a recent study showed that

older adults (60–70 yr) stored ~63% more heat than their younger (20–30 yr) counterparts over the course of four successive 15-min moderate-intensity exercise bouts (each separated by a 15 min recovery). Despite this, the older group exhibited a similar rapid decay in whole body heat loss (Larose, Wright, et al., 2013). Moreover, during and post-exercise suppression of heat loss responses remained intact with successive exercise bouts despite a progressively greater residual heat load measured at end exercise, a heat load which was substantially more elevated in the older adults (Larose, Wright, et al., 2013).

The whole-body sweating rate of an adult male is approximately 40% greater than that of a prepubertal boy, reflecting a larger output per gland and greater gland sensitivity to thermal stimuli. Consequently, children rely to a greater extent on cutaneous blood flow for convective heat loss during exercise. At a given work level, heat production per kg of body mass is inversely related to body size–a disadvantage in small child that is offset by a greater surface area:mass ratio. In addition, the rate of heat acclimatization is slower in children than adults (Rowland, 2008).

3.15.3 Role of Body composition and Gender on Body Temperature

It is well known that body composition can influence peripheral heat loss and Tsk, that the distribution of BF is affected by gender is well known (Eduardo Borba Neves, Salamunes, de Oliveira, & Stadnik, 2017). Body temperature is influenced by a variety of intrinsic factors, such as metabolic rate (M), and changes in health or in body composition may lead to differences in Tsk.

Many early studies comparing male and female response to prolonged exercise, especially in hot environments, concluded that women were more susceptible than men to thermal stress and physical ‘harm’ (Eduardo Borba Neves et al., 2017). Women have a number of physiologic and morphologic characteristics that produce subtle differences in the regulation of BT.

Studies showed that the temperature increase after exercise was higher in the subjects with lower biceps skinfold thickness than in the group with higher biceps

skinfold thickness (Weigert, Nitzsche, Kunert, Lösch, & Schulz, 2018). Therefore, individuals with a low BF% can show higher and faster variations in BT than those with a high BF%. In conclusion, a greater BF% and greater skinfold thickness are associated with lower resting values of Tsk and delayed and lower increases in Tsk during and after exercise.

3.15.4 Role of Metabolic Rate (M)

Metabolic rate can play a significant role in influencing exercise, which elevates Tc at the start of exercise until a new thermal equilibrium is reached. Exercise imparts a large internal heat load caused by the inefficiency with which chemical energy is converted to mechanical work. When performing PE, M increases to satisfy the needs of the human body (Merla et al., 2010). Approximately 30-70% of the energy produced during muscle contraction is dissipated as heat (Bangsbo et al., 2007). Even at maximal mechanical efficiency, more than 75% of the energy liberated during exercise is transferred to the surrounding tissues and environment as heat, while the remainder is used to perform Wk (Cheuvront & Haymes, 2001; González-Alonso et al., 1999b). Therefore, during exercise about 80% of energy consumption is converted into heat with only with about 20% going to the actual work produced by the contraction of the muscles. A high muscle temperature increases the efficiency of muscle contraction (Spencer et al., 2005). However, an excessive rise of BT impairs endurance performance.

Increases in M raise the rate of heat production. Thus, with decreases in Tsk or Tc occurring subsequent to cold stress, thermoregulation is supported by elevating heat production and cutaneous vasoconstriction, the latter of which only attenuates the rate of heat loss. An increase in the rate of metabolic heat production can be evoked via increases in nonshivering or shivering responses. NST is activated by the sympathetic nervous system (Schlader & Vargas, 2019). The primary end-organ for NST is brown adipose tissue, which possesses uncoupling proteins that, upon sympathetic activation, elevate heat production (17). Shivering thermogenesis evokes increases in heat production occurring secondary to involuntary muscle contraction (Mercer, 2001), which are initially fueled by increases in CHOox and increased FATox during more

prolonged cold stress. Shivering is brought about by activation of motor neurons recruited via activation of the sympathetic nervous system (Schlader & Vargas, 2019).

Metabolic Energy

Heat is an inevitable by the product of the inefficiencies in the body's metabolic reactions and muscle movements. The rate of metabolic energy expenditure refers to the rate of free energy released from the catabolism of CHO, fat, and amino acids to resupply ATP for cellular activities such as biosynthesis, transport, and muscular contractions. In oxidizing carbohydrates and fats to carbon dioxide and water during ATP production, and transferring the ATP produced to the functional systems of the cells, about 75% of the original chemical potential energy appears as heat. Except for excreted energy or that used to perform physical work, the remaining 25% of the original energy is also converted to heat when ATP is utilized in the numerous metabolic reactions of the body.

Since metabolic energy is derived primarily through oxidative pathways at rest and during exercise of low-to-moderate intensity (i.e., below the LT), M is generally proportional to the rate of VO_2. Estimates of whole-body M are typically performed using indirect calorimetry based on steady-state VO_2 and the non-protein RER, which accounts for calorific differences between CHO and FAT (Murgatroyd et al., 1993). Metabolic energy is ultimately converted into one of two forms: mechanical energy to perform external work and thermal energy (heat). In the laboratory, Wk rate is regulated using an ergometer or estimated from BM, movement velocity, and the angle of incline during weight-bearing exercise (Snellen, 1960). By combining indirect calorimetry and ergometric values, the rate of metabolic heat production can be estimated as the difference between the rates of metabolic energy expenditure and Wk.

During exercise, greater heat production arises from the increase in VO_2 required to meet the energy demands of the active musculature. Some metabolic energy is converted to useful Wk during activities such as cycling or rowing, but Wk output is negligible during many weight-bearing exercises, e.g. running on a flat surface. Uphill walking and running lead to a net vertical displacement of body mass against gravity

and thus result in a positive external work rate and a greater rate of heat production compared to level-grade walking/running at the same velocity (Bobbert, 1960).

Although heat production rises with exercise intensity, the amount of heat produced at any absolute intensity will vary with mechanical efficiency or movement economy, that is, the ability to transform metabolic energy into useful work or movement. Gross efficiency refers to the percentage of whole-body metabolic energy expenditure that is converted to external work, and typically equals ≤ 25% during cycling or rowing (Fukunaga et al., 1986; Gaesser & Brooks, 1975; Moseley et al., 2004). During weight-bearing activities, movement economy is the mass-specific oxygen cost of moving 1 km ($ml \cdot kg^{-1} \cdot km^{-1}$) correlative VO2 ($ml \cdot kg^{-1} \cdot min^{-1}$) at a specific velocity. Running economy usually ranges from 180 to 220 $ml \cdot kg^{-1} \cdot km^{-1}$, depending on a variety of physiological and biomechanical factors (Barnes & Kilding, 2015; Daniels, 1985; Saunders et al., 2004). Extraordinary values in elite male and female runners of 150 $ml \cdot kg^{-1} \cdot km^{-1}$ (Lucia et al., 2008) and 165 $ml \cdot kg^{-1} \cdot km^{-1}$ (Jones, 2006), respectively, have also been reported.

3.15.5 Role of Dehydration

Dehydration during prolonged exercise in the heat is known to exacerbate cardiovascular strain, increase Tc and impair endurance performance compared with when fluids are ingested during exercise (Fritzsche & Coyle, 2000; González-Alonso et al., 1999b; González-Alonso et al., 1999a; Latzka et al., 1997; Pitts et al., 1944; Sawka & Wenger, 1988). Dehydration can markedly impair cardiovascular function and lead to more pronounced elevations in Tc, which is particularly emphasized in the heat when the requirements for evaporation are maximized.

Relatively few studies have compared the effects of active dehydration to euhydration on human thermoregulatory responses under controlled conditions. Pitts et al., (1944) was the first to systematically demonstrate that active dehydration resulted in a progressive and continued rise in Tc during extended treadmill marches in the heat. The same experiments showed convincingly how both modest and complete replacement of fluid losses attenuated the hyperthermia of dehydration. Costill et al.,

(1970) measured the Tc responses of runners during 2 hours of treadmill running (≈ 25°C) with and without fluid replacement. The observed rise in Tc was ameliorated beyond 1 hour of exercise when fluid was taken.

The integrity of the thermoregulatory system during exercise is logically determined by the maintenance of adequate blood volume. Body water losses (dehydration) increase thermoregulatory strain. The methods used to create a fluid deficit often vary and include diuretic administration, food and fluid restriction, pre-experiment exercise and passive thermal exposure before exercise. These studies are of little practical application for athletes who are adequately hydrated (euhydrated), well nourished, rested and otherwise prepared for competition. Additionally, the method of water loss is known to differently affect the partitioning of fluid between the intracellular and extracellular spaces and can produce both volume and osmotic changes that in- dependently affect temperature regulation (Sawka, 1992).

Losses in plasma volume, even in the absence of osmotic changes, can impair heat loss (Cheuvront et al., 2005). The role of osmotic and volume loss factors in the evolution of hyperthermia is also demonstrable and in agreement with a comprehensive review of hypohydration studies (Sawka, 1992). Therefore, it is clear that active dehydration increases thermoregulatory strain under controlled laboratory conditions. Most studies suggest that dehydration is the most influential factor in determining Tc during a running event. All research showing positive relationships between dehydration and Tc incurred a ≥ 3% body weight deficit.

3.16 Skin Temperature during Exercise

Thermogenesis from exercise is associated with large hemodynamic changes involving multiple thermoregulatory processes. These changes are reflected in Tsk response during exercise (Zontak et al., 1998), and represent a good indication of whether physiological mechanisms are functioning properly, which are vital to the maintenance of thermal homeostasis.

Blood circulation

Physical activity naturally increases muscle metabolism, which can lead to a rise in both muscle and BT by the generation of heat. In such circumstances, the body's surface temperatures change as a consequence of thermoregulatory homeostatic mechanisms that attempt to prevent hyperthermia and release excess heat from the body. Although a small amount of the heat produced by the working skeletal muscles is passively conducted by surrounding tissues to the outer skin, the majority of this heat is transferred by con C through the venous blood flowing from these muscles (Périard et al., 2014). This balance is influenced mainly by the responses of Tc, environment temperature and complex relationships between cutaneous vasodilation and sweating, which facilitate heat exchange with the environment (Merla et al., 2005; Wong & Hollowed, 2016). The delivery of heat from the deeper parts of the body to the skin is accomplished primarily by blood circulation (Zontak et al., 1998), which implies that Tsk can be used as an index to predict thermal changes during exercise (H. Liu et al., 2014; Takada et al., 2013).

Conduction/Convection

Depending on the extent of metabolic energy expenditure, catabolic processes lead to the release of thermal energy (Cramer & Jay, 2016), and therefore muscle activity leads to an increase in muscle temperature (Sawka & Wenger, 1988). The heat generated by the muscle is transferred to adjacent layers of the body and can reach the epidermis via the subcutaneous fatty tissue and the dermis, where it is emitted to the environment.

Heat conduction from the muscle to the skin surface is achieved by conduction of the different layers of the body, which are in direct contact. The subcutaneous fat layer between the heat-generating muscles and skin has a major influence on the heat emitted from the skin surface. Furthermore, heat reaches the skin surface through C in the blood and is then released into the environment through conduction the epidermis, which lacks blood vessels. The influence of convection on thermal transport to the skin surface is

largely affected by the degree of perfusion in the skin; decreasing with vasoconstriction and increasing accordingly with vasodilation.

Environment

Skin temperature varies depending on heat exchange between the body and the environment, primarily mediated by enhanced transportation of blood to skin surface (Taylor et al., 2014). Therefore, during exercise, the impact of environmental conditions influences the skin thermoregulatory response and capacity for heat exchange with the environment (Sawka & Wenger, 1988).

Skin Blood Flow

Thermoregulation is the major process that governs SkBF in humans (Boegli et al., 2003) and several studies use SkBF to describe and characterize the skin thermoregulatory response to exercise (Lee et al., 2000; Zontak et al., 1998). Several studies have been proposed to estimate skin thermoregulatory response from SkBF, and analyze the relationship between Tsk and SkBF during exercise (Xu et al., 2013). Kenney and Johnson (1992) found that the modification of cutaneous blood flow during exercise depends on the individual level of vasodilation and vasoconstriction. The vasoconstrictor response decreases Tsk, induced by a reduction in SkBF; on the contrary, the vasodilator response leads to increase Tsk, associated with a substantial increase in SkBF. Accordingly, there is a close link between thermoregulatory vasodilation and increased SkBF (together with sweating), which is essential for heat dissipation during exercise (Smith et al., 2013).

Type of Exercise

Studies suggest that Tsk behavior varies according to the type of exercise, intensity, duration, as well as muscle mass and subcutaneous fat layers (Neves et al., 2015). Skin temperature during exercise could be related to muscular work, which reflects the efficiency in dissipating the heat produced and in turn depends on the circulatory system recruiting level and sweating rate (Chudecka & Lubkowska, 2012; Xu et al., 2013). A

significant reduction in Tsk is observed during or after an incremental workload or intense workload exercise (Chudecka & Lubkowska, 2012; A. Merla et al., 2010a; Torii, Yamasaki, Sasaki, & Nakayama, 1992; Zontak et al., 1998), which can be related to sweat evaporation for heat dissipation during exercise (Chudecka & Lubkowska, 2012; Gerrett et al., 2019).

Differences in the kinetics of Tsk during exercise were observed between trained and untrained subjects (Abate et al., 2013), and a correlation between Tsk and aerobic capacity was suggested (Chudecka & Lubkowska, 2012; Priego Quesada et al., 2015). However, studies of the relationship between level of VO_{2max} and Tsk are still scarce.

In summary, the relationship of skin thermoregulatory response with Tc, SkBF and environment are modulated by several factors, such as sex (Eduardo Borba Neves et al., 2017), an individual's acclimatization state (Périard et al., 2015), environmental conditions (Cheuvront & Haymes, 2001), body composition (Salamunes et al., 2015), aging (Eduardo Borba Neves et al., 2017), circadian rhythms (K. A. Lee, 1988), the wearing of protective clothing (Kenny & McGinn, 2016), hydration status and/or diet (Baillot & Hue, 2015; Palombo et al., 2010), lifestyle (Atkinson et al., 2005), physiological characteristics (De Andrade Fernandes et al., 2014; Ho et al., 1997a), and relevant to this study, physical conditioning (Boegli et al., 2003; Ho et al., 1997a; Eduardo B. Neves et al., 2015).

3.17 Endurance Aerobic Exercise

Aerobic endurance plays a vital role in overall aerobic performance for endurance and team sports. Both sports have to meet intense aerobic and anaerobic demands during exercise, leading to major metabolic and thermodynamic changes. Endurance exercise refers to events lasting 30 min or more, as defined in the PASSCLAIM document (Saris et al., 2003).

Endurance sports are becoming increasingly popular and more people are running half marathons, marathons, ultramarathons, half Ironmans, and even Ironman

competitions, lasting anywhere between 2 and 17hrs. Many events are organized to encourage people to take up endurance sports, and events of 30 min to 2 hrs, which are more manageable for the novice athlete, are also rapidly increasing in popularity. When determining aerobic fitness, VO_{2max} is considered the most important element for endurance athletes. In studies investigating the physiological effects of endurance exercise, running and cycling have been the most commonly used exercise modes. There are, however, a number of different physiologic, metabolic, and ergogenic responses to endurance exercise (ACSM, 2014) (Table 13).

In team sports, like soccer, players have to perform at high intensities at many points throughout a game, although low intensity activities are performed in more than 70% of the game and are primarily aerobic in nature. As such, soccer-specific endurance should be developed so that players are able to perform at the highest level for 90 minutes and even longer (Jemni, et al., 2018).

In the literature, the terms endurance performance and endurance capacity are often used as synonyms. However, endurance capacity refers to the exercise time to volitional fatigue, whereas performance relates to completing a certain task (running a certain distance, cycling a certain amount of work) as fast as possible. Endurance is typically defined as resistance to fatigue. This could be resistance to fatigue during brief intense exercise but also during sub-maximal prolonged exercise of several hours. For this study and we will define endurance as resistance to fatigue during a mode of exercise where the primary cause of fatigue is induced by substrate depletion or central factors. Typically endurance exercise is 30 minutes or longer. When exercise is longer than 4–5 hours we will refer to this exercise as ultra-endurance exercise (Saris et al., 2003).

In order to understand how thermoregulatory adaptations can improve endurance performance, it is important to understand underlying fatigue mechanisms. In other words, what are the factors that cause fatigue during endurance exercise. Studies in the 1960s demonstrated that one of the main factors was substrate depletion (Sawka & Wenger, 1988). Muscle glycogen depletion seemed to coincide with fatigue, and

athletes with high muscle glycogen stores exercised for longer at a given exercise intensity compared to those with low muscle glycogen stores. In order to decrease muscle glycogen breakdown and to delay or prevent muscle glycogen depletion, various methods have been employed to increase FATox at the cost of CHOox (Saris et al., 2003). In addition, it was shown that with CHO ingestion, blood glucose concentrations and high rates of CHOox could be maintained during exercise, resulting in increased time before exhaustion (Gonzalez et al., 2016). It is generally believed that a rapid delivery of carbohydrates may enhance performance because endogenous carbohydrate stores will be spared (Nilsson et al., 1973).

Table 13. Modes of aerobic (Cardiorespiratory Endurance) exercise to improve physical fitness (ACSM, 2018)

Exercise group	Exercise description	Recommend for	Examples
A	Endurance activities requiring minimal skill or physical fitness to perform	All adults	Walking, leisurely cycling, aqui-aerobics, slow dancing
B	Vigorous intensity endurance activities requiring minimal skill	Adults, who are habitually physically active and/or at least average physical fitness	Jogging, running, rowing, aerobics, spinning, elliptical exercise, stepping exercise, fast dancing
C	Endurance activities requiring skill to perform	Adults with acquired skill and/or at least average physical fitness levels	Swimming, cross- country skiing, skating
D	Recreational sports	Adults with a regular exer-cise program and at least average physical fitness	Racquet sports, basketball, soccer, down-hill skiing, hiking

3.17.1 Recovery

Recovery can be defined as the rate at which maximal force/power output is restored to normal after prior fatigue-inducing exercise (Saris et al., 2003). During exercise involving glycogenolysis as the primary source of energy provision spared (Nilsson et al., 1973), the rate of post-exercise lactate elimination is important to the restoration of normal performance level. Post-exercise [La-] elimination can be studied by investigating the time course of muscle lactate elimination as well as the fall of arterial blood lactate during an ~1h time window immediately following a short all-out exercise bout(s), causing muscle and [La-] concentration to substantially increase. The physical activity level during this recovery period must be strictly standardised (rest or well-

controlled low intensity exercise). Especially in ultra-endurance events, recovery becomes an extremely important factor and maintaining energy balance may be an important issue (Saris et al., 2003).

3.17.2 Measuring Endurance Performance

Endurance performance is largely determined by the ability of the athlete to mobilize and oxidize fat and to spare reserves of CHO, and this is most likely susceptible to specific training (Jeukendrup & Achten).

In the scientific literature, we find studies that have measured endurance capacity as the time to exhaustion when exercising at a constant workload or speed. The advantage of this technique is that it is relatively easy to control and the constant workload allows comparison of metabolic and other measurements in an experimental and a control condition. Such designs make it possible to perform metabolic and other relevant measurements and are also a valid performance measurement. Performance trials have been developed for the treadmill (self-paced runs for a fixed distance), intermittent running (Loughborough Intermit- tent Shuttle Run test; Saris et al., 2003), cycling (self-paced time trial), rowing ergometer and various other intermittent sports such as squash and tennis (Saris et al., 2003).

A performance test must be reliable (reproducible) and valid. The validity of a test refers to the extent to which an individual's test performance reflects true performance. Reliability refers to the consistency of performance when an individual performs the test repeatedly (Saris et al., 2003; Hopkins, 2000). Time trials (constant work tests) and constant duration tests are the most reliable measurements and are also likely to be more valid indicators of true performance. However, it must also be noted that it may be easier to detect differences between two experimental protocols using a time to exhaustion test（Saris et al., 2003).

It is crucial that performance tests are conducted under strictly controlled conditions. Heart rate, work rate, speed and time should not be shown to subjects in studies with multiple trials. Experiments should not be conducted in pairs and they

should be performed in quiet labs with no distractions. Trained subjects will generally give more reliable results than untrained subjects, especially when using time trial protocols. A familiarisation trial is necessary as a learning effect is generally observed. Hopkins et al. (2000) reported that performance increased between the first and second trial on average by 1.2% (likely range 0.5 to 1.9%), whereas the increase in subsequent trials was only 0.2% (likely range –0.3 to 0.7%).

3.18 Endurance Training

The extreme physical endurance demands and varied environmental settings of endurance sports have provided a unique opportunity to study the limits of human thermoregulation for more than a century.

Endurance exercise training results in profound adaptations of the cardio-respiratory and neuromuscular systems that enhance the delivery of oxygen from the atmosphere to the mitochondria and enable a tighter regulation of muscle metabolism. These adaptations affect an improvement in endurance performance that is manifest as a rightward shift in the 'velocity-time curve'. This shift enables athletes to exercise for longer at a given absolute exercise intensity, or to exercise at a higher exercise intensity for a given duration. The performance of repeated bouts of exercise over a period of time causes numerous physiological changes that result in improved performance in that exercise activity. The magnitude of the training response depends on the duration of the exercise bouts, their intensity and the frequency with which they are performed along with the initial training status, genetic potential, age and gender of the individual (Mujika, 2016).

Endurance training results in numerous adaptations within skeletal muscle that may be significant for exercise performance, including increases in sodium-potassium pump concentration, lactate transport capacity and possibly myoglobin concentration (Jones & Carter, 2000). Endurance training also results in a marked increase in the oxidative capacity of skeletal muscle, contributing to an increased capacity to oxidize lipid reserves in the muscles, thus reducing mobilization of glycogen stores at submaximal exercise (60-85% VO_{2max}). Therefore, endurance adaptations following chronic

endurance includes improved muscle glycogen storage and glycogen 'sparing' at sub-maximal exercise intensities through increased fat utilization, enhanced lactate kinetics, morphology changes including greater type I fibre proportions per muscle area, and increases in capillary and mitochondrial density (Jones & Carter, 2000).

Therefore, endurance exercise training results in numerous adaptations to the neuromuscular, metabolic, cardio-vascular, respiratory, endocrine and thermoregulatory systems. These adaptations are reflected in improvements in the key parameters of aerobic fitness. These are the VO_{2max}, exercise economy, the lactate/ventilatory threshold and O_2 kinetics (Figure 9). Other parameters that may help determine endurance performance, and that are related to the other 4 parameters, are the velocity at VO_{2max} (V-VO_{2max}) and the maximal lactate steady state or critical power (Jones & Carter, 2000).

3.18.1 Endurance Training and Thermoregulatory System

Regular aerobic training improves thermoregulatory function, leading to improvements in heat dissipation during exercise (Fritzsche & Coyle, 2000; Larose, Wright, et al., 2013; Stapleton et al., 2014), a response which recently has been shown to result in a correspondingly greater rate of Tc recovery (Stapleton et al., 2014). However, this was not paralleled by improvements in the rate of heat dissipation during recovery, which highlights the strong influence of nonthermal intrinsic factors in the modulation of post exercise thermoregulation. Indeed, restoration of body Tc was more rapid in the adults with higher fitness due only to an enhanced capacity to dissipate heat during exercise (Merla et al., 2010b). In contrast to long-term endurance training, the influence of short-term exercise training on thermoregulatory function has yielded mixed findings, with some studies reporting greater heat loss responses of SkBF and sweating, paralleled by reductions in Tc (González-Alonso et al., 1999a; González-Alonso et al., 1999b), whereas others reported no effect (Palombo et al., 2010). One study reported comparable improvements in local heat loss responses (i.e., earlier onset threshold for SkBF and sweating) and Tc (lower resting and end-exercise values) following an 8-wk exercise training program, although this was not paralleled by improvements in whole body heat loss during and following moderate-intensity exercise

in warm ambient conditions (Laursen et al., 2006). Therefore, physical training also increases SkBF at any given increase in core temperature.

Therefore, it does appear that aerobic fitness confers an increased capacity to tolerate higher Tc. However, as recently reported, the lack of an improvement in the body's ability to dissipate heat should be interpreted with caution as differences may only be evident beyond a given heat load (Karlsen et al., 2015) and whether heat acclimation provides a similar benefit remains to be determined (Karlsen et al., 2015).

Endurance training in temperate climates reduces physiological strain and increases exercise capacity in the heat, as endurance-trained athletes exhibit many of the characteristics of heat-acclimated individuals (Périard et al., 2014). However, while physical training by virtue of the thermoregulatory strain can impart some heat acclimation, the requirement of profuse sweating and warm skin is critical. Trained individuals exercising at the same relative intensity, but at a higher metabolic rate as untrained individuals, experience a higher rate of heat storage (Fernández-Elías et al., 2015) and fatigue at a similar (Périard et al., 2014; Sawka, 1992), or higher Tc (Mallette et al., 2016; Selkirk & McLellan, 2001). Hence, adaptations related to training may allow for greater rates of body heat accumulation before a reduction in work rate occurs, be it voluntary or involuntary (Fernández-Elías et al., 2015). In addition, aerobically fit individuals develop heat acclimation more rapidly than their less fit counterparts, and high aerobic fitness might reduce the susceptibility to heat injury/illness (Gardneret al., 1996). It has been estimated that VO_{2max} accounts for approximately 44% of the variability in exercise heat tolerance, and the number of days required for complete development of heat acclimation (Latzka et al., 1997). However, endurance training alone does not totally replace the benefits of heat acclimation produced by a program of exercise in the heat (Périard et al., 2014).

To achieve improved thermoregulation from endurance training in temperate climates, either strenuous interval training or continuous training at an exercise intensity greater than 50% VO2max should be employed. Lesser training intensities produce questionable effects on performance during exercise heat stress (Périard et al., 2014).

The endurance training must last at least 1 week (Lorenzo et al., 2010) and some authors show that the greatest improvements require 8–12 weeks of training (Périard et al., 2014).

3.18.2 Skin Temperature and Aerobic Fitness

During endurance exercise, Tsk plays a fundamental role in thermoregulatory processes. During exercise, the response and relationship of skin thermoregulatory response to Tc, SkBF and the environment are modulated by important factors, such as physical fitness.

Aerobic fitness level is an important determinant in the health status of individuals of any age. It was reported that VO_{2peak} decreases about 7% per decade (Wilson & Tanaka, 2000). Based on this discovery, the decrease in skin thermoregulatory capacity, associated with reduced SkBF, may be related to the decline in VO_{2peak} (Stapleton et al., 2014). However, other studies reported that fitness level, associated with regular endurance-type exercise, can induce partial acclimation (Périard et al., 2014; Selkirk & McLellan, 2001) and thereby improve the ability to thermoregulate, enhancing the skin vasodilation response during exercise (Lee et al., 2000; Shibasaki et al., 2010). Boegli (2003) concluded that endurance training modifies the skin thermoregulatory response, as manifested by a greater augmentation of skin perfusion and maintenance of active cutaneous vasodilation during exercise (Lee et al., 2000; Shibasaki et al., 2010). Other studies (Lee et al., 2000; Shibasaki et al., 2010) also reported that physical endurance training and increased VO_{2peak} improve skin thermoregulatory response, which appears to be one of the main elements needed for an effective thermoregulatory active vasodilation response, as well as increased SkBF (Ely et al., 2009; Stapleton et al., 2014). Moreover, Richmond et al., (2014) found that there was an association between enhanced SkBF response and increased VO_{2peak} after exercise training in older subjects. These changes in VO_{2peak} seemed to affect Tsk responses. Périard et al. (2014) also postulated that a better skin thermoregulatory response during endurance exercise could indicate a higher cardiorespiratory fitness level, despite aging.

In recent years, the measurement of Tsk has played an important role, and interest in Tsk adaptations during and after endurance exercise has increased since studies have shown that endurance exercise leads to an increase in Tsk (Périard et al., 2014; Richmond et al., 2014; Michael N. Sawka et al., 2012; Stapleton et al., 2014). Despite these discoveries, Tsk response remains unstudied as an independent parameter in the control of BT during exercise, and it is unclear if the level of aerobic fitness can influence Tsk response, irrespective of age (Best et al., 2014; Shellock & Prentice, 1985).

4. AIMS AND HYPOTHESIS

4.1 Aims

The main purpose of the project was to analyze and compare the dynamic of Tsk during exercise in different populations with different metabolic responses to exercise. For this purpose, it was necessary to carry out two studies:

1. A first study analyzed an compared the correlation between Tsk and cardiorespiratory variables during an incremental maximal stress test in high fit (HF) and moderately fit (MF) male endurance runners.

2. A second study analyzed and compared the correlation between Tsk and metabolic flexibility by measuring [La^-] along with FATox and CHOox rates during an incremental maximal stress test in highly trained (HT) competitive endurance runners and moderately active (MA) male runners, and professional soccer (PS) players.

The secondary aims of the present project were:

1. To analyze the dynamic of Tsk response in different populations during an incremental exercise test to VO_{2peak} on an ergometer treadmill.

2. To analyze the five minutes recovery period after the exercise tests in both studies, to monitor any responses that may take place.

3. To determine the skin thermoregulatory response as related to thermal changes during exercise. Measuring Tsk could offer indirect hemodynamic information of the vasodilation response during exercise-related thermal adjustment.

4. To determine the skin thermoregulatory response as related to the level of aerobic fitness during an incremental maximal exercise test.

5. To determine if the validated exercise protocol of Achten (2003) can provide a measurement of maximal rate of Fat oxidation (MFO), and to assess the correlation with the thermoregulatory response.

6. To determine the correlation between $VO_{2peak,}$, [La^{-}] concentrations, FATox and CHOox rates, skin thermoregulatory response, RPE values, and Fat % and muscle Mass % in HT endurance runners, MA runners and professional soccer players during an incremental maximal exercise test.

4.2 Hypothesis

These aims were based on the following working hypotheses:

First study:

1. We hypothesized that higher aerobic capacity could be associated with an enhanced Tsk response in male endurance runners.

2. We hypothesized that HF endurance runners have the ability to maintain a higher Tsk response than MF runners during maximal exercise.

3. We hypothesized that Tsk measurement can indirectly provide hemodynamic information of the vasodilation response during exercise-related thermal adjustment, and can be used to predict the vasodilatation response.

4. We hypothesized that Tsk response can be used as an index to predict aerobic fitness.

Second study:

1. We hypothesized that higher metabolic flexibility could be associated with an enhanced Tsk response in highly trained endurance and moderately active runners and professional soccer players.

2. We hypothesized that HT endurance runners have the ability to maintain higher Tsk rates than MA runners and PS players during absolute submaximal exercise intensities.

3. We hypothesized that HT endurance runners have higher metabolic flexibility, and therefore higher oxidative and lactate clearance capacities.

5. MATERIAL AND METHODS

5.1 First Study

5.1.1 Study Design and Participants

The present study is a cross-sectional observational study that analysed the cardiorespiratory behavior related to Tsk in men who were highly and moderately fit endurance runners.

A total of 89 fit male endurance runners participated in this study, recruited from running and triathlon teams in the Barcelona city area, who were divided into two groups depending on their fitness level. They all performed progressive exercise tests to maximal oxygen consumption (VO_{2peak}) on a treadmill. Participants were eligible for the study if they met the following inclusion criteria: all subjects had to be at an aerobic fitness level ≥ 40th percentile, based on the American College of Sports Medicine (ACSM) age-specific cardiorespiratory fitness classification (ACSM, 2018); participate in regular running training, at least three times/week and a minimum of 120-150 min/week; have competed in an endurance event (> 5km) within the 3 months prior to the study; and have ≥3 years of competitive running or triathlon experience (Saris et al., 2003). All subjects were nonsmokers, deemed healthy (assessed by completion of a general health questionnaire), with no known cardiovascular or metabolic disorders, and were not taking medication that had the potential to impact cardiovascular or thermoregulatory function. All significant inclusion criteria were the same for all runners, except age, which was between 18 and 50 yrs.

Participants were divided into two groups: highly fit endurance runners (HF) and moderately fit runners (MF). The HF (n = 35; age 36 ± 8 yrs) was >80th percentile and the MF (n = 44; age 37 ± 9 yrs) was 80th percentile based on the ACSM age-specific cardiorespiratory fitness classification (ACSM, 2018) (Table 7).

After the initial screening (Appendix II), 10 were not enrolled because of severe exercise contraindications, or use of medicines/supplements that may have an effect on

their thermoregulatory and physical response to exercise. This left a total of 79 endurance runners that met the study criteria for participation in the study.

Subjects were instructed to maintain their training routines throughout the experimental period and to refrain from intense exercise for 48 hours before testing.

5.1.2 Ethical Concerns

The present study respects the ethical principles of (Amaro et al, 1996; Gillon, 1994) non maleficence, beneficence, autonomy, justice and confidentiality. It also followed the guidelines of the World Medical Association (2006), which regulates the obligatory nature of informed consent in clinical investigations, as it is a PA study but with the application of sports medical tests. The procedures of this study followed the Helsinki guidelines (WMA, 2016) for ethical behavior and was approved by the local Human Research Ethics Committee of Blanquerna, University of Ramon Llull (Ethical Code: 1819006D).

Therefore, all participants signed an informed consent form prior to participation. All subjects were informed of the purpose of this study, its risks and procedures. At a meeting, the project was explained and all questions were addressed. After signing the informed consent form, a health screening questionnaire was completed by each participant (Appendix II).

5.1.3 Testing Procedures

All subjects participated in familiarization sessions prior to testing at the lab facilities, even though each participant had previously done between 1 to 3 maximal stress tests to measure VO_{2max} on a treadmill.

Because external and internal factors can influence performance on the day of the test, and in order to measure Tsk under similar conditions, the following characteristics were used as exclusion criteria and participants were asked not to: a) smoke or drink

alcohol at least 12h before the test; b) sunbathe or expose themselves to UV rays 24h before the test c) use body lotions or creams 24h before the test; d) carry out high-intensity or exhaustive exercise less than 24h before the test; e) eat at least 2h before the test and refrain from having a heavy meal; f) drink coffee or stimulants 2h before the test; and g) use medications, such as antipyretics or diuretics, or any dietary supplement that could potentially interfere with water homeostasis and body temperature in the two weeks prior to the test. Each participant was measured at a similar time in order to reduce the intra-subject effect of the circadian cycle. Finally, as heat acclimation can influence overall control of Tsk during exercise (Karlsen et al., 2015), we decided to perform our study in winter and spring, avoiding the possible effects of heat acclimation during the warmer season.

Before the test, all participants underwent a medical screening to evaluate possible contraindications (Appendix II). This was followed by body composition measurements (Appendix III). Afterwards, participants completed an incremental maximal treadmill test (Appendix III). Tsk and cardiovascular data were also continuously monitored during the exercise test, followed by a recovery period of five minutes.

5.1.4 Anthropometric Measurements

Body mass was measured to the nearest 0.1 kg on a digital scale (Seca 861, Hamburg, Germany), with the subject wearing lightweight clothing and no shoes. Body height was measured using a stadiometer to the nearest 0.1 cm (Seca 225, Seca, Hamburg, Germany). Body mass index (BMI) (kg/m^2) was calculated using body mass and body height, following the recommendations of the International Society for the Advancement of Kinanthropometry (Marfell-Jones, M.J., Olds, T., Stewart, A.D. and Carter, 2006).

Body density was estimated using the seven site skinfold equation (chest, axilla, subscapular, midaxillary, triceps, abdominal and thigh) developed by Jackson and Pollock (Jackson & Pollock, 1978). Skinfold measurements were taken on the right side from the average of the measurements three obtained by the same researcher using a Holtain skinfold caliper (Holtain Ltd., Walles, UK) and following the International

Society for the Advancement of Kinanthropometry guidelines (Garber et al., 2011). Body fat percentage (%) was calculated using Siri's equation (1961), with muscle mass percentage determined thereafter. Muscle mass percentage (%) was determined together with bone and organs percentages using the equation of the sum of seven perimeters (arm, contracted arm, forearm, wrist, chest, upper thigh, medial thigh and calf) and 6 diameters (biacromial iliac spine, breadth, chest, humerus, femur, anterior-posterior thoracic and transverse thoracic) (Drinkwater, D.T. and Ross, 1980). Bone mass % was calculated using Rocha's equation, residual mass % was calculated using Wurch's equation and fat-free mass % was determined thereafter (Esparza Ros, 1993).

5.1.5 Cardiorespiratory Fitness Assessment

All tests were performed in the morning between 9am and 12pm to reduce the intra-subject effect of the circadian cycle. The test was carried out in a controlled environment, where conditions were maintained at 22 ± 1 ºC and 50 ± 5% relative humidity.

Each participant performed progressive an incremental maximal stress test on a treadmill (Quasar model, HP Cosmos sports & medical gmbh, Nussdorf-Traunstein, Germany). All cardiorespiratory variables, the rate of perceived exertion (RPE) and Tsk were monitored at rest, during exercise and during the 5 min recovery period

At rest and during exercise values of cardio-vascular and ventilation responses were monitored using a gas analyzer. The following parameters were obtained:

- Absolute oxygen consumption (VO_2, $L \cdot min^{-1}$): liters of O_2 consumed per minute and its value at rest is ~0.22 $L \cdot min^{-1}$ (López Chicharro & Izquierdo Redín, 2006; Wilmore & Costill, 2004).

- Relative oxygen consumption (VO_2, $mL \cdot kg \cdot min^{-1}$): oxygen uptake with respect to body weight in milliliters of oxygen consumed per minute per kilogram of body weight.

- Respiratory exchange ratio (RER): is the relationship between the volume of CO_2 produced (VCO_2) and the volume of O_2 consumed ($VCO_2 \cdot VO_2^{-1}$) (López Chicharro & Izquierdo Redín, 2006; Wilmore & Costill, 2004).

- Carbon dioxide production (VCO_2, $L \cdot min^{-1}$ STPD): the amount of CO_2 produced.

- Ventilation (VE, $L \cdot min^{-1}$): the basic functions of pulmonary ventilation are O_2 and CO_2 exchange with the environment, which regulates blood pH and oral communication. The ventilation level is regulated by the respiratory center as a function of metabolic needs, the gaseous state, the acid-base balance of the blood, and the mechanical conditions of the lung-thorax. The purpose of ventilation is to transport O_2 to the alveolar space so the exchange at the pulmonary capillary space is done and the CO_2 produced at a metabolic level is evacuated. In a maximal exercise test, these values indicate the magnitude of the response of the lung function, showing the amount of air exchanged per minute. The values at rest are ~ 6 $L \cdot min^{-1}$ (López Chicharro & Izquierdo Redín, 2006; Wilmore & Costill, 2004).

All these variables were measured breath-by-breath with an automatic gas analysis system (Ergospirometer, Metalyzer 3B; Cortex-medical, Leipzig, Germany) equipped with a pneumotachometer and a two-way mask (Hans Rudolph, Kansas, USA). Gas and volume calibrations were performed before each test, according to the manufacturer's guidelines. The system's volume and gas analysers were calibrated using a 3 litre calibration pump and calibration gas (15.12% o2; 5.10% CO_2), respectively. VO_{2peak} was calculated as the average oxygen uptake over the last 30s of the test. Subjects were required to wear the mask to collect respiratory gases, which were averaged every 10s throughout the entire test, and then used to calculate substrate metabolism (López Chicharro & Izquierdo Redín, 2006; Wilmore & Costill, 2004).

The twelve lead electrocardiograms (CardioScan v.4.0, DM Software, Staline, Nevada, USA) and heart rate (HR) (Polar RS800CX, Polar Electro, Lake Success, New

York) were monitored continuously during the test and during the five minute recovery period.

The test was determined to be maximal if two of the following four criteria were met: 1) a levelling off of VO_{2max} with further increases in workload (< 2 ml ^{x} kg^{-1} body mass), 2) a HR within 10 beats ^{x} min^{-1} of age predicted maximum (220 beats ^{x} min^{-1} minus age), 3) RER exceeded >1.05, respiratory gas measurements (VO_2 and VCO_2), or 4) a rating of perceived exertion (RPE) of >17.

As exercise starts, HR increases directly in proportion to the increase in the intensity of the exercise up to a point close to exhaustion. Some authors suggest that the linear relationship holds up submaximal HRs around 170 beats·min-1, and from this point HR tends to increase slowly and approach asymptotically at a maximum value (López Chicharro & Izquierdo Redín, 2006). Achieving the theoretical maximum HR is a criterion for maximality of the stress test. There are many equations for calculating maximum HR based on age. The most commonly used is 220 beats minus the age in years of the subject. However, it should be kept in mind that this equation is only an approximation and that individual values can vary considerably (Wilmore, J., & Costill, 2004).

Finally, the rating of perceived exertion (RPE) on the Borg scale (Borg et al., 1987) was recorded during the last 15 s of each exercise stage.

Testing Protocol

In this study, each participant performed an incremental exercise test to obtain VO_{2max} on a treadmill ergometer. During the test, participants started at a speed of 7 km/h^{-1}, which increased by 1 km/h^{-1} every 2 min until exhaustion with a maximal speed reached, followed by a recovery period of five minutes. The participants performed the test at a constant slope (1.5%). In this study, all cardiorespiratory variables, the rate of perceived exertion (RPE) and Tsk were monitored at rest, during exercise and during the recovery period.

5.1.6 Skin Temperature Assessment

On the day of the test, subjects reported normal hydration. Skin temperature was continuously recorded with a Tsk sensor (thermistor sensor, TSD202D, Biopac Systems Inc., Goleta, CA, USA) that was placed on the left pectoralis muscle 2.5 cm medial and 2.5 cm above the nipple. Accuracy and precision of the device model is ± 0.2 C. Before monitoring Tsk, participants were acclimated to the environment by standing in the room for 15 min. During the incremental test, Tsk data was recorded every half second and the mean value of each 10s was used for data analysis (Biopac Student Lab Analysis software, Biopac Systems Inc., Goleta, CA, USA).

5.1.7 Statistical Analysis

Descriptive statistics were calculated for all variables. To test the normality of the variables the Kolmogorov-Smirnov test was applied. Experiment data are presented as means ± SED in all tables unless stated otherwise.

In the first study, a one-way ANOVA with post-hoc Bonferroni test was used to determine between-group differences. Pearson's r correlation was used to analyze the associations between Tsk and cardiorespiratory variables. Finally, a multiple linear regression identifying significant variables was developed with Tsk used as the response variable. The explanatory variables were those found to be significantly correlated ($p < 0.05$) with Tsk, based on a linear relationship. Multicollinearity was checked with the variance inflation factor (VIF), which needed to be below 10 for all predictor variables
(Field, 2009).

5.2 Second Study

5.2.1 Study Design and Participants

The present study is a cross-sectional observational and comparative study that analyses the metabolic behaviors related to Tsk in three different populations: highly trained competitive male endurance runners, moderately active male runners, and professional male soccer players.

A total of 22 highly trained competitive male endurance runners (HT), 20 moderately active male runners (MA) and 23 professional male soccer players (PS) participated in this study, performing graded exercise tests to obtain VO_{2peak} on a treadmill. All participants from the HT, MA and SP groups were eligible for the study if they met the following inclusion criteria: all subjects had to have an aerobic fitness level ≥40th percentile based on the American College of Sports Medicine (ACSM) age-specific cardiorespiratory fitness classification (ACSM, 2018), be nonsmokers, be deemed healthy (based on responses to a general health questionnaire), have no known cardiovascular or metabolic disorders, and not be on medication with the potential to impact cardiovascular or thermoregulatory function.

Both HT endurance and MA runners were recruited for the study from running and triathlon teams in the Barcelona city area. Members of the HT group ranged in competitive level from club/county standard to elite endurance runners; had a maximal oxygen uptake (VO_{2max}) of 55-65 $ml \cdot kg^{-1} \cdot min^{-1}$; had competed in an endurance event (> 5km) within 3 months prior to the study; and had ≥3 years of competitive running or triathlon experience (Saris et al., 2003; Zourdos et al., 2012). For the MA group, the requirements were to exercise at least three times/week with a minimum of 120-150 min/week.

For the SP group, the requirement for participation was placement on a national soccer team in the professional spanish league system, operated by the Spanish Football Federation. In this case, we recruited soccer players from a club in the 2ºB professional league.

Fifty participants were recruited for the HT and MA runners groups from various running and triathlon teams in the Barcelona city area. After the initial screening (Appendix II), 8 runners from these clubs were not enrolled because of severe exercise contraindications or use of medicines/supplements that may have an important effect on their thermoregulatory and physical response to exercise. This left a total of 22 highly trained/competitive endurance runners and a total of 20 moderately active runners that met the criteria for participation in the study.

Twenty-seven PS players were recruited for this study from a professional soccer team in the 2ºB professional Spanish league. After the initial screening (Appendix II), 4 soccer players were not enrolled because of the use of medicines/supplements that may have a large effect on their thermoregulatory and physical responses to exercise. Furthermore, both goalkeepers were unable to participate, which left a total of 23 PS players that met the criteria for participation in the study. The soccer players participated in this study during the soccer season, in winter and spring.

In this study, Tsk, cardiovascular data, substrate oxidation rates (FATox and CHOox), and [La^-] measurements were continuously monitored during an incremental exercise test, followed by a recovery period of five minutes.

5.2.2 Ethical Concerns

The present study respects the ethical principles of (Amaro et al, 1996; Gillon, 1994) non maleficence, beneficence, autonomy, justice and confidentiality. It also followed the guidelines of the World Medical Association (2006), which regulates the obligatory nature of informed consent in clinical investigations, as it is a PA study but with the application of sports medical tests. The procedures of this study followed the Helsinki guidelines (WMA, 2016) for ethical behavior and was approved by the local Human Research Ethics Committee of Blanquerna, University of Ramon Llull (Ethical Code: 1819006D).

Therefore, all participants signed an informed consent form prior to participation. All subjects were informed of the purpose of this study, its risks and procedures. At a

meeting, the project was explained and all questions were addressed. After signing the informed consent form, a health screening questionnaire was completed by each participant (Appendix II).

5.2.3 Testing Procedures

All subjects participated in familiarization sessions prior to testing at the lab facilities, even though each participant had previously done between 1 to 3 maximal stress tests to measure VO_{2max} on a treadmill.

Because external and internal factors can influence performance on the day of the test, and in order to measure Tsk under similar conditions, the following characteristics were used as exclusion criteria and participants were asked not to: a) smoke or drink alcohol at least 12h before the test; b) sunbathe or expose themselves to UV rays 24h before the test c) use body lotions or creams 24h before the test; d) carry out high-intensity or exhaustive exercise less than 24h before the test; e) eat at least 2h before the test and refrain from having a heavy meal; f) drink coffee or stimulants 2h before the test; and g) use medications, such as antipyretics or diuretics, or any dietary supplement that could potentially interfere with water homeostasis and body temperature in the two weeks prior to the test. Each participant was measured at a similar time in order to reduce the intra-subject effect of the circadian cycle. Finally, as heat acclimation can influence overall control of Tsk during exercise (Karlsen et al., 2015), we decided to perform our study in winter and spring, avoiding the possible effects of heat acclimation during the warmer season.

Before the test, all participants underwent a medical screening to evaluate possible contraindications (Appendix II). This was followed by body composition measurements (Appendix III). Afterwards, participants completed an incremental maximal treadmill test. Tsk, cardiovascular and metabolic data were also continuously monitored during the exercise test, followed by a recovery period of five minutes. For all participants did he same test was repeated with all groups subjects.

5.2.4 Anthropometric Measurements

Body mass was measured to the nearest 0.1 kg on a digital scale (Seca 861, Hamburg, Germany), with the subject wearing lightweight clothing and no shoes. Body height was measured using a stadiometer to the nearest 0.1 cm (Seca 225, Seca, Hamburg, Germany). Body mass index (BMI) (kg/m^2) was calculated using body mass and body height, following the recommendations of the International Society for the Advancement of Kinanthropometry (Marfell-Jones, M.J., Olds, T., Stewart, A.D. and Carter, 2006).

Body density was estimated using the seven site skinfold equation (chest, axilla, subscapular, midaxillary, triceps, abdominal and thigh) developed by Jackson and Pollock (Jackson & Pollock, 1978). Skinfold measurements were taken on the right side from the average of the measurements three obtained by the same researcher using a Holtain skinfold caliper (Holtain Ltd., Walles, UK) and following the International Society for the Advancement of Kinanthropometry guidelines (Garber et al., 2011). Body fat percentage (%) was calculated using Siri's equation (1961), with muscle mass percentage determined thereafter. Muscle mass percentage (%) was determined together with bone and organs percentages using the equation of the sum of seven perimeters (arm, contracted arm, forearm, wrist, chest, upper thigh, medial thigh and calf) and 6 diameters (biacromial iliac spine, breadth, chest, humerus, femur, anterior-posterior thoracic and transverse thoracic) (Drinkwater, D.T. and Ross, 1980). Bone mass % was calculated using Rocha's equation, residual mass % was calculated using Wurch's equation and fat-free mass % was determined thereafter (Esparza Ros, 1993).

5.2.5 Cardiorespiratory Fitness Assessment

All tests were performed in the morning between 9am and 12pm to reduce the intra-subject effect of the circadian cycle. The test was carried out in a controlled environment, where conditions were maintained at 22 ± 1 ºC and 50 ± 5% relative humidity.

Each participant performed progressive an incremental maximal stress test on a

treadmill (Quasar model, HP Cosmos sports & medical gmbh, Nussdorf-Traunstein, Germany). All cardiorespiratory variables, substrates oxidation rates (FATox and CHOox), [La^-] measurements, rating of perceived exertion (RPE) and Tsk were monitored at rest, during exercise and during the 5 min recovery period

At rest and during exercise values of cardio-vascular and ventilation responses were monitored using a gas analyzer. The following parameters were obtained:

- Absolute oxygen consumption (VO_2, $L \cdot min^{-1}$): liters of O_2 consumed per minute and its value at rest is ~0.22 $L \cdot min^{-1}$ (López Chicharro & Izquierdo Redín, 2006; Wilmore & Costill, 2004).

- Relative oxygen consumption (VO_2, $mL \cdot kg \cdot min^{-1}$): oxygen uptake with respect to body weight in milliliters of oxygen consumed per minute per kilogram of body weight.
- Respiratory exchange ratio (RER): is the relationship between the volume of CO_2 produced (VCO_2) and the volume of O_2 consumed ($VCO_2 \cdot VO_2^{-1}$) (López Chicharro & Izquierdo Redín, 2006; Wilmore & Costill, 2004).

- Carbon dioxide production (VCO_2, $L \cdot min^{-1}$ STPD): the amount of CO_2 produced.

- Ventilation (VE, $L \cdot min^{-1}$): the basic functions of pulmonary ventilation are O_2 and CO_2 exchange with the environment, which regulates blood pH and oral communication. The ventilation level is regulated by the respiratory center as a function of metabolic needs, the gaseous state, the acid-base balance of the blood, and the mechanical conditions of the lung-thorax. The purpose of ventilation is to transport O_2 to the alveolar space so the exchange at the pulmonary capillary space is done and the CO_2 produced at a metabolic level is evacuated. In a maximal exercise test, these values indicate the magnitude of the response of the lung function, showing the amount of air exchanged per minute. The values at rest are ~ 6 $L \cdot min^{-1}$ (López Chicharro & Izquierdo Redín, 2006; Wilmore & Costill, 2004).

All these variables were measured breath-by-breath with an automatic gas analysis system (Ergospirometer, Metalyzer 3B; Cortex-medical, Leipzig, Germany) equipped with a pneumotachometer and a two-way mask (Hans Rudolph, Kansas, USA). Gas and volume calibrations were performed before each test, according to the manufacturer's guidelines. The system's volume and gas analysers were calibrated using a 3 litre calibration pump and calibration gas (15.12% o2; 5.10% CO_2), respectively. VO_{2peak} was calculated as the average oxygen uptake over the last 30s of the test. Subjects were required to wear the mask to collect respiratory gases, which were averaged every 10s throughout the entire test, and then used to calculate substrate metabolism (López Chicharro & Izquierdo Redín, 2006; Wilmore & Costill, 2004).

The twelve lead electrocardiograms (CardioScan v.4.0, DM Software, Staline, Nevada, USA) and heart rate (HR) (Polar RS800CX, Polar Electro, Lake Success, New York) were monitored continuously during the test and during the five minute recovery period.

The test was determined to be maximal if two of the following four criteria were met: 1) a levelling off of VO_{2max} with further increases in workload (< 2 ml ^{x} kg^{-1} body mass), 2) a HR within 10 beats ^{x} min^{-1} of age predicted maximum (220 beats ^{x} min^{-1} minus age), 3) RER exceeded >1.05, respiratory gas measurements (VO_2 and VCO_2), or 4) a rating of perceived exertion (RPE) of >17.

As exercise starts, HR increases directly in proportion to the increase in the intensity of the exercise up to a point close to exhaustion. Some authors suggest that the linear relationship holds up submaximal HRs around 170 beats·min-1, and from this point HR tends to increase slowly and approach asymptotically at a maximum value (López Chicharro & Izquierdo Redín, 2006). Achieving the theoretical maximum HR is a criterion for maximality of the stress test. There are many equations for calculating maximum HR based on age. The most commonly used is 220 beats minus the age in years of the subject. However, it should be kept in mind that this equation is only an approximation and that individual values can vary considerably (Wilmore, J., & Costill, 2004).

Finally, the rating of perceived exertion (RPE) on the Borg scale (Borg et al., 1987) was recorded during the last 15 s of each exercise stage.

Testing Protocol

A total of 65 male participants (22 highly trained competitive endurance runners, 20 moderately active runners, and 23 professional soccer players) performed an incremental maximal exercise test. Each participant was measured at a similar time in order to reduce the intra-subject effect of the circadian cycle. Anthropometric characteristics (body height and mass) were collected before the test. Participants performed an incremental maximal exercise test with 3-min stages, followed by a recovery period of 5 min. The interval duration in the Fat_{max} test was selected based on a previous study (Achten & Jeukendrup, 2003a,b, 2004). Tsk, FATox and CHOox (Achten et al., 2003) and [La^{-}], measurements were taken.

In this study, each participant performed an incremental exercise test to obtain VO_{2max} on a treadmill ergometer. During the test, participants started at an initial speed of 5.0 km/h^{-1} at a gradient of 1,5% for 1 min, after which the speed increased to 9 km/h^{-1}, then increased by 1 km/h every 3 min until exhaustion with a maximal speed reached, followed by a recovery period of five minutes. The exercise protocol used here was adapted from a previously described and validated protocol on treadmill (Mohebbi & Azizi, 2011) in which it was concluded that an incremental exercise test with 3-min stages could be used to determine both MFO and FAT_{max} (Achten & Jeukendrup, 2003a,b, 2004).

In this study all cardiorespiratory variables, substrate oxidation rates, metabolic responses, the rate of perceived exertion (RPE), and Tsk were monitored at rest, during exercise and during the recovery period. We used indirect calorimetry (FATox, CHOox) and [La^{-}] measurements to assess metabolic flexibility during exercise across populations

5.2.6 Calculations of Fat and Carbohydrate Oxidation

We used indirect calorimetry and [La-] measurements to study the metabolic responses to exercise in HT runners, MA runners, and PS players.

Values of VO_2 consumption and VCO_2 production were averaged for each exercise stage. The raw data was then analyzed manually for each athlete. Breath-by-breath data were averaged in 10s increments for the maximal cardiopulmonary test, and 60s average values were used to calculate substrate oxidation in the Fat_{max} test. For each of these stages, substrate oxidation (FATox and CHOox) rates were calculated by using stoichiometric equations applied according to the methodology described by Frayn (1983), with the assumption that urinary nitrogen excretion rate was negligible. FATox and CHOox rates were calculated as follows:

- FATox ($g^{x}\ min^{-1}$) = 1.67 x VO_2 ($L.min^{-1}$) – 1.67 x VCO_2 ($L.min^{-1}$)

- CHOox ($g^{x}\ min^{-1}$) = 4.55 x VCO_2 ($L.min^{-1}$) – 3.21 x VO_2 ($L.min^{-1}$)

(Frayn, 1983)

FATox and CHOox rates were calculated for each stage by averaging the data from the last 1 minute of each stage, and the stage with the highest level of FATox was recognized as Fat_{max}. FATox and CHOox rates were then plotted as a function of exercise intensity, expressed as a percentage of maximal oxygen uptake (VO_{2peak}). From each fat oxidation curve, several features were identified according to a previously described procedure (Achten J, 2003): 1) MFO, the peak rate of fat oxidation measured over the entire range of exercise intensities, 2) FAT_{max}, the exercise intensity at which the FATox rate was maximal, and 3)FAT_{min}: the exercise intensity where the fat oxidation rate becomes negligible and reached zero (i.e., where RER >1.0).

5.2.7 Lactate Concentration Measurement

At the end of each stage of the test, around the last 30 seconds, a capillary blood sample was collected from the finger and used to analyze blood lactate concentration (Lactate Plus). Measurements of [La^-], together with FATox and CHOox, provide an

indirect method to assess metabolic flexibility and oxidative capacity during the test for all groups (San-Millán & Brooks, 2017).

5.2.8 Skin Temperature Assessment

On the day of the test, subjects reported normal hydration. Skin temperature was continuously recorded with a Tsk sensor (thermistor sensor, TSD202D, Biopac Systems Inc., Goleta, CA, USA) that was placed on the left pectoralis muscle 2.5 cm medial and 2.5 cm above the nipple. Accuracy and precision of the device model is ± 0.2 C. Before monitoring Tsk, participants were acclimated to the environment by standing in the room for 15 min. During the incremental test, Tsk data was recorded every half second and the mean value of each 10s was used for data analysis (Biopac Student Lab Analysis software, Biopac Systems Inc., Goleta, CA, USA).

5.2.9 Statistical Analysis

Descriptive statistics were calculated for all variables. To test the normality of the variables the Kolmogorov-Smirnov test was utilized. Experimental data are presented as means ± SEM in all tables unless stated otherwise.

In this study, a one-way ANOVA with post-hoc Bonferroni test for multiple comparisons were used to determine the statistical significance of mean values observed in the three groups at baseline, at the end of the test and the end of the recovery period. Pearson's r correlation coefficients were used to assess the statistical significance of the relationships among the variables studied.

Statistical significance was set at $p < 0.05$. Statistical analyses were conducted using the Statistical Package for the Social Sciences (IBM SPSS, v 22.0, Chicago, IL, USA).

6. RESULTS

6.1 First Study

A total of 79 male participants (35 high fit endurance runners; 44 moderately fit runners; (Table 14) performed an incremental maximal exercise test. Participants had participated in familiarization sessions on they already performed previously other maximal stress exercise test on a treadmill. Measurements to every participant were obtained at a similar time in order to reduce the intra-subject effect of the circadian cycle. Anthropometric characteristics (body height and mass) were collected before the test, the participants performed an incremental maximal test, where the speed was increased every 2 min until exhaustion, followed by a recovery period of 5 mins.

6.1.1 Participants Characteristics and Cardiorespiratory Assessments

A total Table 14 shows general and physiological characteristics of all participants. All subjects were similar in height and age. Body weight and BMI were significantly higher in the MF group (p = .001), compared with the subjects of the HF group. The HF group had a higher muscle mass % and a lower fat mass % than the MF group (all p < .001).

Table 14. Physical characteristics of the whole sample and of the two study groups separately

Variables	n=79	HF (n=35)	MF (n=44)	*p*-value
Characteristics				
Age (years)	36 ± 9	36 ± 8	37 ± 9	.734
Height (cm)	177 ± 0.1	176 ± 0.5	178 ± 0.6	.189
Weight (kg)	75.62 ± 7.56	72.72 ± 5.67	77.93 ± 8.12	**.001**
BMI (kg/m^2)	24.03 ± 2.11	23.23 ± 1.50	24.68 ± 2.32	**.001**
Fat mass (%)	14.04 ± 4.52	11.69 ± 3.2	15.91 ± 4.59	**< .001**
Muscle mass (%)	45 ± 3.48	46.51 ± 2.70	43.80 ± 3.61	**< .001**
VO_{2peak} (L/min)	3.82 ± 0.38	4.02 ± 0.35	3.65 ± 0.33	**.001**
VO_{2peak} (ml/kg/min)	51.74 ± 6.54	56.62 ± 4.31	47.86 ± 5.29	**< .001**
HR (beat/min)	181 ± 9	182 ± 8	181 ± 11	.554
RER (VCO_2/VO_2)	1.05 ± 0.05	1.05 ± 0.05	1.04 ± 0.05	.590
VE (L/m)	136 ± 20	145 ± 21	129 ± 17	**< .001**
$Speed_{peak}$ (km/h)	15.91 ± 1.78	16.98 ± 1.50	15.06 ± 1.50	**< .001**
Time spent (sec)	1200 ± 212	1329 ± 181	1099 ± 180	**< .001**

Note: values are means ± SD. Abbreviations: BMI (body mass index), VO_{2peak} (peak oxygen consumption), HR (heart rate), RER (respiratory exchange ratio), VE (ventilation), $Speed_{peak}$ (peak speed), HF (highly fit endurance runners), MF (moderately fit endurance runners).

Cardiovascular response

VO_{2peak} was significantly higher in the HF group than the MF group (56.62 ± 4.31 vs. 47.86 ± 5.29 ml/kg/min, $p < 0.05$). Also, both the maximal speed reached during the test and VE were higher in the HF group compared with the MF group (all p < .001). Significant differences in peak HR (HR_{peak}) and peak RER (RER_{peak}) were not found.

6.1.2 Skin Temperature Measurements

Results from the analysis of Tsk are shown in Table 15. These results provide the mean Tsk responses at rest (baseline), during exercise, and after exercise during the recovery period. Baseline Tsk was significantly higher (p = .049) in the HF group compared with the MF group.

Table 15. Skin temperature response in an incremental exercise test until volitional exhaustion in high and moderately fit endurance runners

Variables	n=79	HF (n=35)	MF (n=44)	*p*-value
$Tsk_{baseline}$ (°C)	34.06 ± 0.75	34.24 ± 0.84	33.91 ± 0.74	**.049**
Tsk_{peak} (°C)	36.03 ± 0.73	36.20 ± 0.60	35.90 ± 0.79	.062
Tsk_{final} (°C)	35.59 ± 0.95	35.70 ± 0.77	35.50 ± 1.07	.322
Tsk variation from $Tsk_{baseline}$ to Tsk_{peak} (°C)	1.97 ± 0.81	1.96 ± 0.65	1.99 ± 0.92	.833
Tsk variation from $Tsk_{baseline}$ to Tsk_{final} (°C)	1.53 ± 1.03	1.49 ± 0.84	1.56 ± 1.16	.747
Tsk variation from Tsk_{peak} to Tsk_{final} (°C)	- 0.45 ± 0.49	- 0.50 ± 0.50	- 0.41 ± 0.49	.413
Time-duration from $Tsk_{baseline}$ to Tsk_{peak} (sec)	996 ± 240	1105 ± 244	910 ± 201	< **.001**
Time-duration from Tsk_{peak} to Tsk_{final} (sec)	204 ± 163	223 ± 159	189 ± 167	.348
Tsk_{peak} during recovery (°C)	36.25 ± 0.81	36.38 ± 0.79	36.14 ± 0.81	.187
Tsk_{final} during recovery (°C)	36.12 ± 0.88	36.28 ± 0.87	36 ± 0.88	.167
Tsk variation from Tsk_{final} at end of exercise to Tsk_{peak} during recovery (°C)	0.71 ± 0.59	0.76 ± 0.56	0.68 ± 0.61	.557
Tsk variation from Tsk_{final} at end of exercise to Tsk_{final} at end of recovery (°C)	0.60 ± 0.68	0.65 ± 0.56	0.54 ± 0.75	.452

Note: values are means ± SD. Abbreviations: Tsk (skin temperature), $Tsk_{baseline}$ (baseline skin temperature), Tsk_{peak} (maximal skin temperature), Tsk_{final} (skin temperature at the end of exercise/recovery), HF (highly fit endurance runners) and MF (moderately fit endurance runners).

Values of Tsk at each percentage of peak workload during the incremental test are shown in Figure 12. Mean Tsk and standard error (SEM) of both groups together during exercise are shown in Figure 12. This figure also shows mean Tsk and SEM for each

group during the recovery period. During the test, Tsk values were higher in the HF group compared with the MF group (Figure 12). However, these were not statistically significant differences. As the duration and intensity of the test increased, the maximal Tsk (Tsk_{peak}) reached was lower in the MF group compared with the HF group throughout exercise (35.90 ± 0.79 vs 36.20 ± 0.60 ºC, respectively). Nevertheless, there were not statistically significant differences between groups (Figure 12). Similarly, during the recovery period, the peak values of Tsk were higher in the HF group compared with the MF group (Figure 12). Both groups reached their Tsk_{peak} during recovery. However, there were not statistically significant differences between groups.

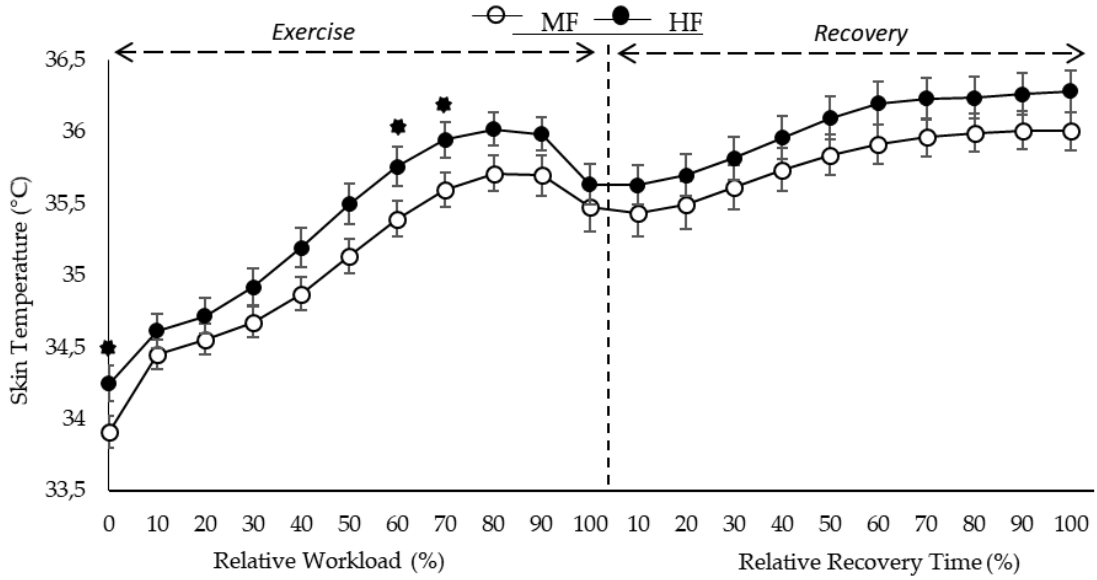

Figure 12. Values are means ± SEM. The black circles represent the highly fit endurance runners (HF) group and the white circles represent the moderately fit endurance runners (MF) group. Significant level was set at $p < 0.05$. Analysis was performed at the end of each relative workload of exercise and recovery period. Significant differences between highly and moderately fit endurance runners.

During the test, irrespective of Tsk_{peak} and fitness level, the increase of Tsk from $Tsk_{baseline}$ to Tsk_{peak} was not statistically different (1.96 ± 0.65 vs. 1.99 ± 0.92 ºC in HF and MF, respectively) during the test. The difference between the Tsk at the beginning of the recovery period and the higher Tsk achieved in this period for both groups was not statistically different (0.65 ± 0.56 vs 0.54 ± 0.75 ºC in HF and MF, respectively) (Figure 12).

Figure 12 also illustrates the mean responses of Tsk for each group during the recovery period, in which there were not significant differences between groups. The comparison of percentage workload (%) of Tsk responses in the HF and MF subjects during the test and recovery period are shown in Figure 12. Throughout the exercise period, Tsk response for the HF group was significantly higher at baseline (p = .049), at 60% (p = .048) and 70% (p = .048) of peak workload (%) compared with the MF group (Figure 13). There were no other differences in Tsk among the groups during test and recovery periods (Figure 13). The mean values of Tsk at the end of the test were slightly higher in the HF group, nevertheless, the difference among groups was not significant (HF 35.70 ± 0.77 vs MF 35.50 ± 1.07 ºC). During the incremental test both groups reached stable Tsk values (plateau) at 80 to 90% of peak workload (Figure 12). After reaching the plateau, Tsk started decreasing in both groups (HF = 0.50 ± 0.50 vs LF = 0.41 ± 0.49 ºC). The Tsk_{peak} during the recovery period was greater than the Tsk_{peak} reached during exercise in both groups (HF 36.38 ± 0.79 ºC vs MF 36.14 ± 0.81 ºC, respectively), and was also greater than the final Tsk value after recovery.

As showed in Table 16, Tsk_{peak} of both group was inversely correlated with fat mass %. On the other hand, Tsk was positively correlated with age, muscle mass %, VO_{2peak}, HR_{peak}, VE_{peak} ($p < 0.05$) and $Speed_{peak}$ (p = .002) (Table 16).

Table 16. Correlation Coefficients and p-values among Tsk_{peak} and Age, BMI, Fat mass %, Muscle mass %, VO_{2peak}, HR_{peak}, RER_{peak}, VE_{peak} and $Speed_{peak}$.

Variables	Correlation coefficient	p-value
Age, years	.306	.006
BMI (kg/m^2)	-.147	.196
Fat mass (%)	-.276	.014
Muscle mass (%)	.263	.019
VO_{2peak} (ml/kg/min)	.299	.007
HR_{peak} (beat/min)	.286	.011
RER_{peak}	-.035	.760
VE_{peak} (L/min)	.256	.023
$Speed_{peak}$ (km/h)	.337	.002

Note: values are means ± SD. Abbreviations: Tsk_{peak} (peak skin temperature), BMI (body mass index), VO_{2peak} (peak oxygen consumption), HR_{peak} (peak heart rate), RER_{peak} (peak respiratory exchange ratio), VE_{peak} (peak ventilation) and $Speed_{peak}$ (peak speed).

The multivariate linear regression that was used to identify factors that significantly affected Tsk_{peak} showed that $Speed_{peak}$ had a significant effect on Tsk_{peak}. Although HR_{peak} has positive effects on Tsk_{peak}, no significant difference was found between groups for this variable (Table 17).

Table 17. Variables that affect Tsk_{peak}

Variables	β	β error	*t*	*p*	VIF
(Constant)	31.668	1.453	21.790	< .001	-
$Speed_{peak}$	.111	.046	2.392	.019	1.139
HR_{peak}	.014	.008	1.694	.094	1.138

Regression model statistically significant $p = .002$; $R^2 = 0.146$.
Abbreviations: Tsk_{peak} (peak skin temperature), VIF (variance inflation factor); $Speed_{peak}$ (peak speed) and HR_{peak} (peak heart rate).

6.2 Second Study

6.2.1 Participants Characteristics and Cardiorespiratory Assessments

The physical characteristics of the participants are shown in Table 18. A total of 65 male participants (22 high trained endurance runners; 20 moderately active runners; 23 professional soccer player; (Table 18) performed an incremental maximal exercise test. Participants had participated in familiarization sessions on they performed previously other maximal stress exercise test on a treadmill. Measurements for every participant were obtained at a similar time in order to reduce the intra-subject effect of the circadian cycle. Anthropometric characteristics (body height and mass) were collected before the test, the participants performed an incremental maximal stress exercise test on a treadmill with stages of 3–min duration, followed by a recovery period of 5 mins.

Table 18. General characteristics of the whole sample

Variables	n	Mean	Minimum	Maximum	SD
Age, years	65	31.38	19	45	7.03
Height (cm)	65	177.59	160.00	194.00	7.00
Weight (kg)	65	74.01	57.00	89.90	6.96
BMI (kg/m^2)	65	23.47	19.84	27.08	1.45
Fat mass (%)	65	10.26	6.55	19.04	2.12
Muscle mass (%)	65	46.90	40.67	53.06	2.51
VO_{2peak} (ml/kg/min)	65	53.80	39.45	62.50	5.27
HR_{peak} (beat/min)	65	181.42	161	200	8.37
RER_{peak}	65	1.07	0.96	1.20	0.44
VE_{peak} (L/min)	65	142.09	99.00	197.00	21.42
$Speed_{peak}$ (km/h)	65	16.09	12.00	18.00	1.51

Note: values are means ± SD. Abbreviations: BMI (body mass index), VO_{2peak} (peak oxygen consumption), HR_{peak} (peak heart rate), RER_{peak} (peak respiratory exchange ratio), VE_{peak} (peak ventilation) and $Speed_{peak}$ (peak speed).

Cardiovascular

The anthropometric, cardiopulmonary and metabolic responses of each group to the maximal test are given in Table 19. All subjects were similar in height and muscle mass %. BMI and fat mass % were significantly lower in the HT and PS groups compared with the subjects from the MA group (p = .001 and p < .001, respectively). The MA group had a lower weight than the HT and PS groups (all p = .017).

VO_{2peak} was significantly higher in the HT group than in the PS group (58.57 ± 2.33 vs. 53.34 ± ml/kg/min, $p < 0.05$) and the MA group (49.07 ± 4.67 ml/kg/min, $p < 0.05$). Maximal speed reached during the test was significantly higher in HT than PS ($p < .001$) and MA groups ($p < .001$) (Table 19). Significant differences in peak heart rate (HR_{peak}), peak RER (RER_{peak}) and VE were not found.

Table 19. Anthropometrics and cardiorespiratory characteristics of the three study groups.

Variables	HT (n=22)	MA (n=20)	PS (n=23)	*F*	*p*-value
Anthropometric					
Age (years) *; **; ***	33 ± 5	37 ± 5	25 ± 4	41.484	**< .001**
Height (cm)	176 ± 6.7	176 ± 7.8	181 ± 5.7	3.828	**.027**
Weight (kg) **	70.68 ± 6.67	75.65 ± 7.41	75.94 ± 5.80	4.374	**.017**
BMI (kg/m^2) *; ***	22.86 ± 1.33	24.39 ± 1.48	23.25 ± 1.16	7.457	**.001**
Fat mass (%) *; ***	9.19 ± 1.38	12.11 ± 2.32	9.66 ± 1.39	17.106	**< .001**
Muscle mass (%)	47.75 ± 2.09	45.96 ± 3.33	46.90 ± 1.75	2.823	.067
Cardiorespiratory					
VO_{2peak} (L/min) *; **; ***	4.14 ± 0.33	3.7 ± 0.53	4.03 ± 0.33	6.899	**.002**
VO_{2peak} (ml/kg/min) *; **; ***	58.57 ± 2.33	49.07 ± 4.67	53.34 ± 3.67	35.901	**< .001**
HR (beat/min)	181 ± 9	183 ± 8	180 ± 7	0.662	.520
RER (VCO_2/VO_2)	1.07 ± 0.03	1.08 ± 0.05	1.06 ± 0.05	1.320	.275
VE (L/m)	148 ± 16	135 ± 24	142 ± 23	1.897	.159
$Speed_{peak}$ *; **; ***	17.41 ± 0.80	14.45 ± 1.14	16.26 ± 0.81	54.552	**< .001**

Note: values are means ± SD. Abbreviations: BMI (body mass index), VO_{2peak} (peak oxygen consumption), HR (heart rate), RER (respiratory exchange ratio), VE (ventilation), $Speed_{peak}$ (peak speed), HT (high trained endurance runners), MA (moderately active runners) and PS (professional soccer players). * Significant difference ($p \leq 0.05$) between HT vs. MA. ** Significant difference ($p \leq 0.05$) between HF vs. PS. *** Significant difference ($p \leq 0.05$) between MA vs PS.

6.2.2 Skin Temperature Measurements

Results from the analysis of Tsk of all groups are shown in the Figure 13. These results provide the mean Tsk responses at rest (baseline) and during exercise of all groups. The mean value of the $Tsk_{baseline}$ was significantly lower in the MA group compared with SP group (33.86 ± 0.54 vs. 34.20 ± 0.42 ºC, $p < 0.05$) (Figure 13; Table 20). Tsk_{peak} was significantly higher in both HT and PS groups (36.68 ± 0.68 vs. 36.96 ± 0.42 ºC, $p < 0.01$) compared with the MA group (35.97 ± 0.66 ºC, $p < 0.01$) (Figure 13; Table 20). There were not significant differences in the Tsk_{final} at the end of the recovery period between groups.

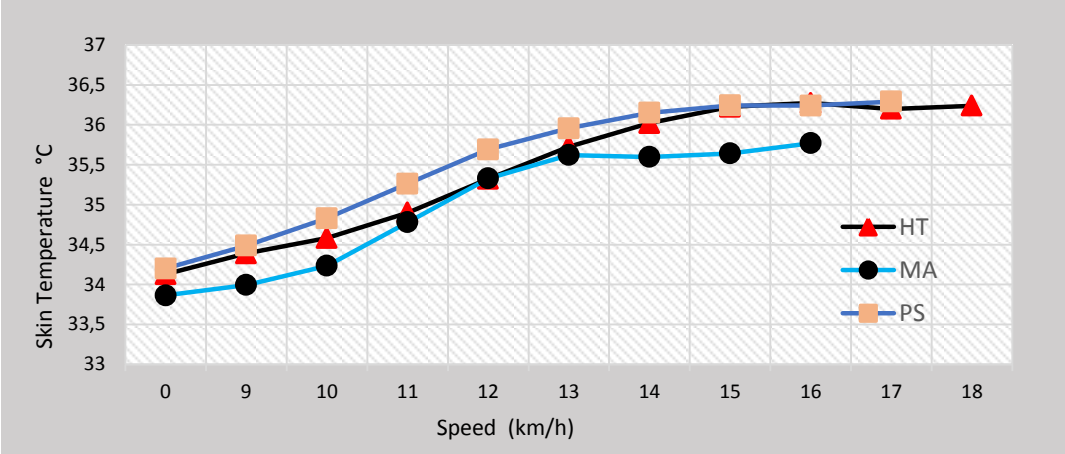

Figure 13. Relationships between average rates of Tsk and exercise speed in HT endurance runners, MA runners, and PS players. Note: values are means. Abbreviations: HT high trained endurance runners, MA moderately active healthy runners, PS professional soccer players.

Table 20. Metabolic and skin temperature data of the three study groups.

Variables	HT (n=22)	MA (n=20)	PS (n=23)	*F*	*p* -value
Metabolic					
$FATox_{baseline}$ (g·min) ***	0.19 ± 0.05	0.14 ± 0.06	0.21 ± 0.09	4.915	**.010**
$FATox_{peak}$ (g·min) **	0.63 ± 0.13	0.51 ± 0.23	0.49 ± 1.17	3.935	**.025**
$FATox_{final}$ (g·min) during recovery	0.03 ± 0.03	0.01 ± 0.02	0.03 ± 0.05	2.144	.126
$CHOox_{baseline}$ (g·min)	0.10 ± 0.09	0.17 ± 0.11	0.16 ± 0.16	1.515	.228
$CHOox_{peak}$ (g·min)	6.07 ± 0.83	5.76 ± 1.20	5.72 ± 0.98	0.811	.449
$CHOox_{final}$ (g·min) during recovery	1 ± 0.33	1.10 ± 0.36	0.96 ± 0.33	0.417	.661
$[La^{-}]_{baseline}$ (mmol·L ^{1})	1.12 ± 0.40	1.16 ± 0.39	1.13 ± 0.34	0.068	.934
$[La^{-}]_{peak}$ (mmol·L ^{1}) ***	7.19 ± 1.21	8.25 ± 1.90	7.08 ± 1.22	3.963	**.024**
$[La^{-}]_{final}$ (mmol·L ^{1}) during recovery ***	6.99 ± 1.36	7.77 ± 2.00	6.23 ± 1.59	4.554	**.014**
Skin Temperature					
$Tsk_{baseline}$ (°C) ***	34.13 ± 0.34	33.86 ± 0.54	34.20 ± 0.42	3.428	**.039**
Tsk_{peak} (°C) *; ***	36.68 ± 0.68	35.97 ± 0.66	36.96 ± 0.79	10.786	< **.001**
Tsk_{final} (°C) during recovery	36.51 ± 0.53	36.31 ± 0.59	36.32 ± 0.66	0.746	.478

Note: values are means ± SD. Abbreviations: $FATox_{baseline}$ (baseline fat oxidation), $FATox_{peak}$ (maximal fat oxidation), $FATox_{final}$ (final fat oxidation), $CHOox_{baseline}$ (baseline carbohydrate oxidation), $CHOox_{peak}$ (maximal carbohydrate oxidation), $CHOox_{final}$ (final carbohydrate oxidation), $[La^{-}]_{baseline}$ (baseline blood lactate concentrations), $[La^{-}]_{peak}$ (maximal blood lactate concentrations), $[La^{-}]_{final}$ (final blood lactate concentrations) $Tsk_{baseline}$ (baseline skin temperature), Tsk_{peak} (maximal skin temperature), HT (high trained endurance runners), MA (moderately active runners) and PS (professional soccer players). * Significant difference ($p \leq 0.05$) between HT vs. MA. ** Significant difference ($p \leq 0.05$) between HF vs. PS. *** Significant difference ($p \leq 0.05$) between MA vs PS.

6.2.3 Substrates Oxidation Rates (FATox and CHOox)

During the treadmill test, results from the analysis of FATox and CHOox of all groups are shown in the Figure 14. These results provide the mean FATox and CHOox

rates at rest (baseline) and during exercise of all groups. The mean value of $FATox_{baseline}$ rates was significantly higher in the PS group compared with MA (0.21 ± 0.09 vs. 0.14 ± 0.06 ºC, $p < 0.05$) (Figure 14; Table 20). However, MFO oxidation or $FATox_{peak}$ was significantly higher in the HT group compared with the SP group (0.63 vs. 0.49 $g \cdot min^{-1}$, $p < 0.05$) but we did not find differences with the MA group (0.63 vs. 0.51 $g \cdot min^{-1}$). Fat_{max} oxidation occurred at an exercise intensity around 64 ± 3%; 60 ± 4.2%; and 59 ± 5.1% VO_{2peak} in HT, MA and PS groups, respectively. There were not significant differences in the $FATox_{final}$ at the end of the recovery period between groups (Table 20). There were no significant differences in CHOox rates between groups (Figure 15, Table 20).

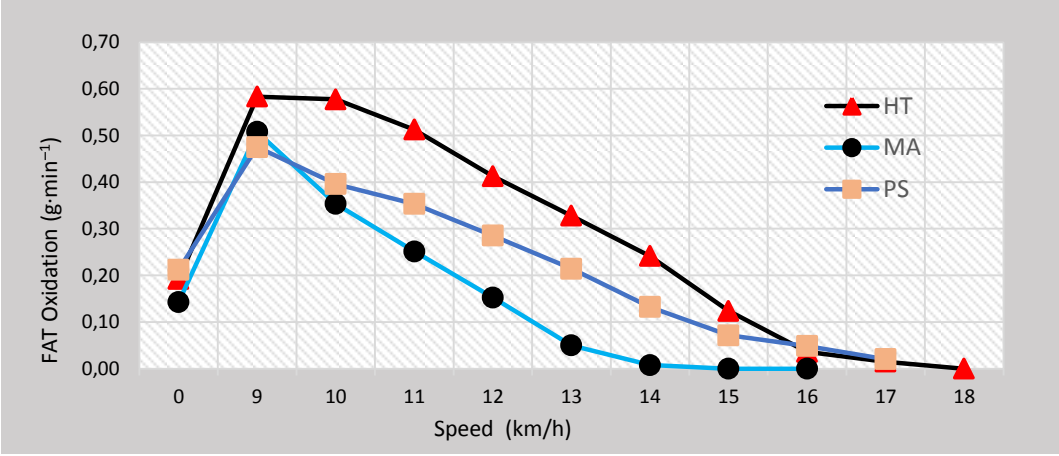

Figure 14. Relationships between average rates of FATox and exercise speed in HT endurance runners, MA runners, and PS players. Note: values are means. Abbreviations: HT high trained endurance runners, MA moderately active healthy runners, PS professional soccer players.

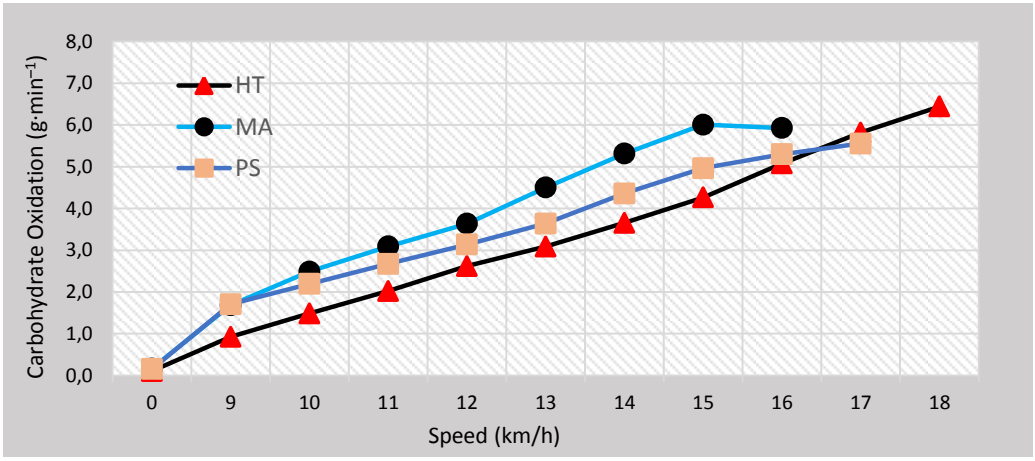

Figure 15. Relationships between average rates of CHOox and exercise speed in HT endurance runners (HT), MA runners, and PS players. Note: values are means. Abbreviations: HT high trained endurance runners, MA moderately active healthy runners, PS professional soccer players

6.2.4 Blood Lactate Response

Figure 16 shows the blood lactate values during the incremental maximal exercise used. These results provide the mean [La^-] concentrations at rest (baseline) and during exercise of all groups. Resting lactate concentrations, $[La^-]_{baseline}$ were similar for all groups (Table 20). Blood lactate concentrations were significantly higher in the MA group at the end of the test $[La^-]_{peak}$ compared with the PS group (8.25±1.90 vs. 7.08±1.22 mmoL/L^{-1}, $p < 0.05$). At the end of the test, HT group and PS group had similar maximal [La^-] concentrations (7.19±1.21 vs. 7.08±1.22 mmoL/L^{-1}), respectively (Table 20; Figure 16). At the end of recovery period, PS players showed significantly lower [La^-] concentrations compared with MA runners (6.23±1.59 vs. 7.77±2 mmoL/L^{-1}, $p < 0.05$).

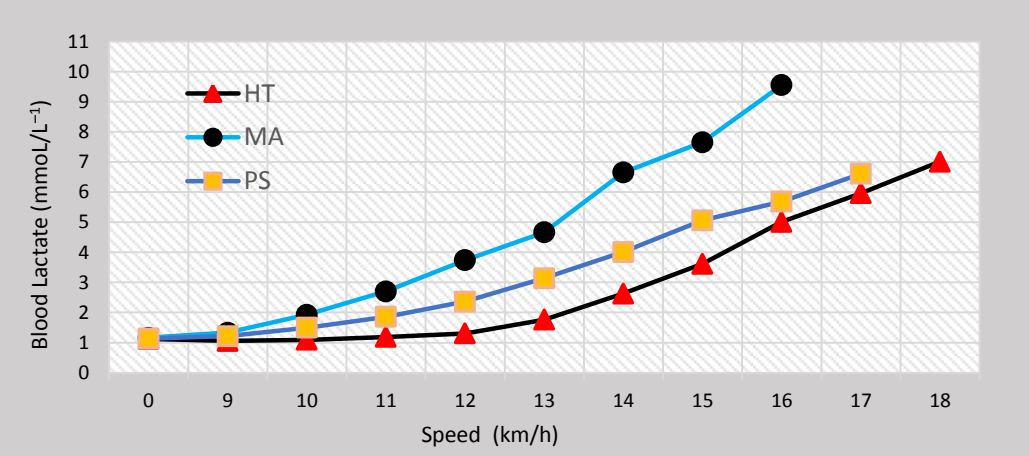

Figure 16. Relationships between average blood lactate levels and exercise speed in HT endurance runners, MA runners, and PS players. Note: values are means. Abbreviations: HT high trained endurance runners, MA moderately active healthy runners, PS professional soccer players.

Figure 17 shows the correlations between [La^-] concentrations and Tsk for all data points of the HT group (A); MA group (B) and PS group (C). Significant correlations were found between [La^-] and Tsk for each group (HT: $r = 0.572$, $p < 0.01$; MA: $r = 0.589$, $p < 0.01$; PS: $r = 0.603$, $p < 0.01$).

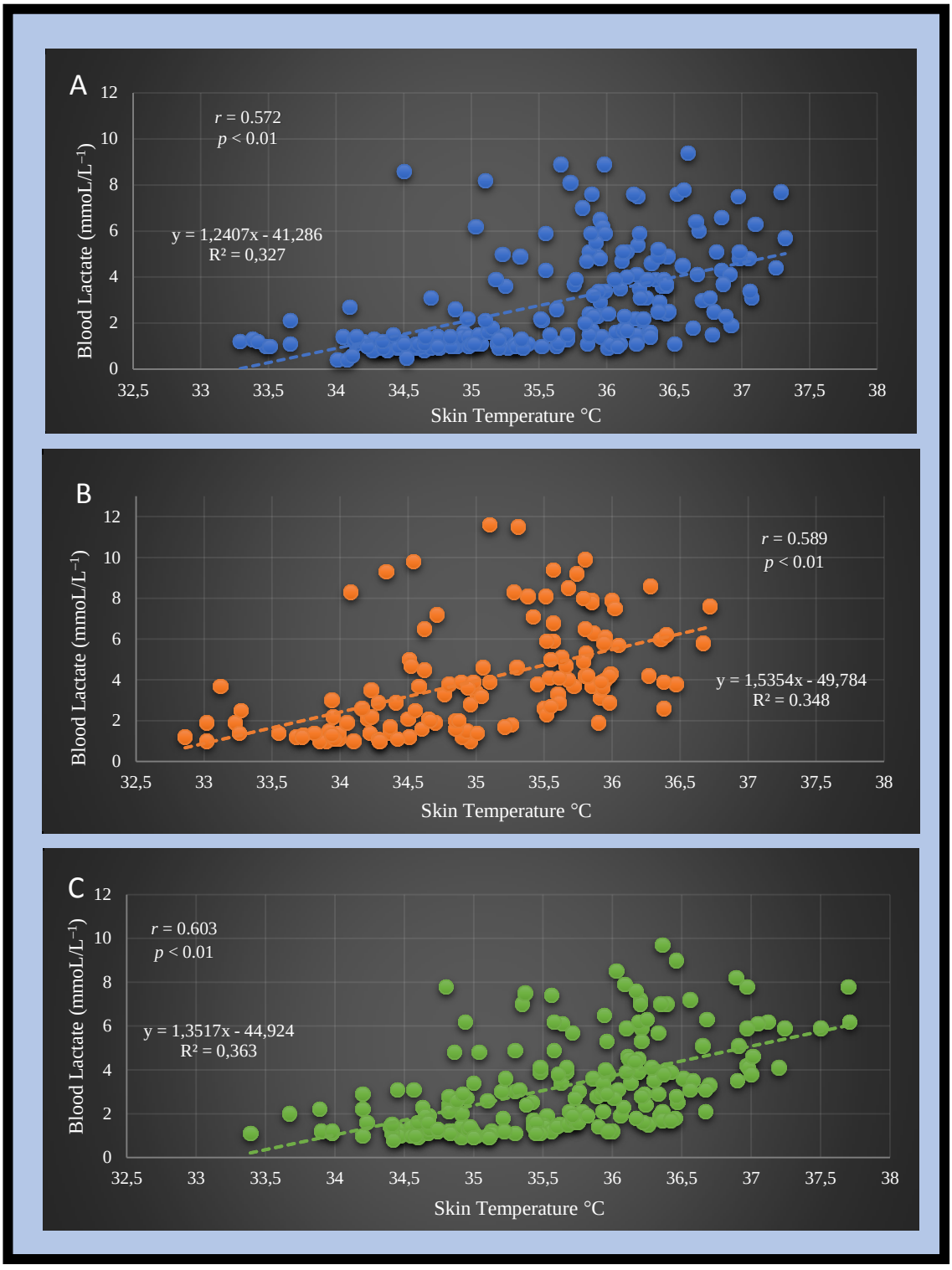

Figure 17. Relationships between blood lactate and skin temperature for all data points from **A)** high trained endurance runners, **B)** moderately active healthy runners, and **C)** professional soccer players.

Figure 18 shows the correlations between FATox rates and Tsk for all data points of the HT group (A); MA group (B) and PS group (C). Significant correlations were found between FATox rates and Tsk for each group (HT: r = - 0.497, p < 0.01; MA: r = -0.430, p < 0.01; PS: r = -0.378, p < 0.01).

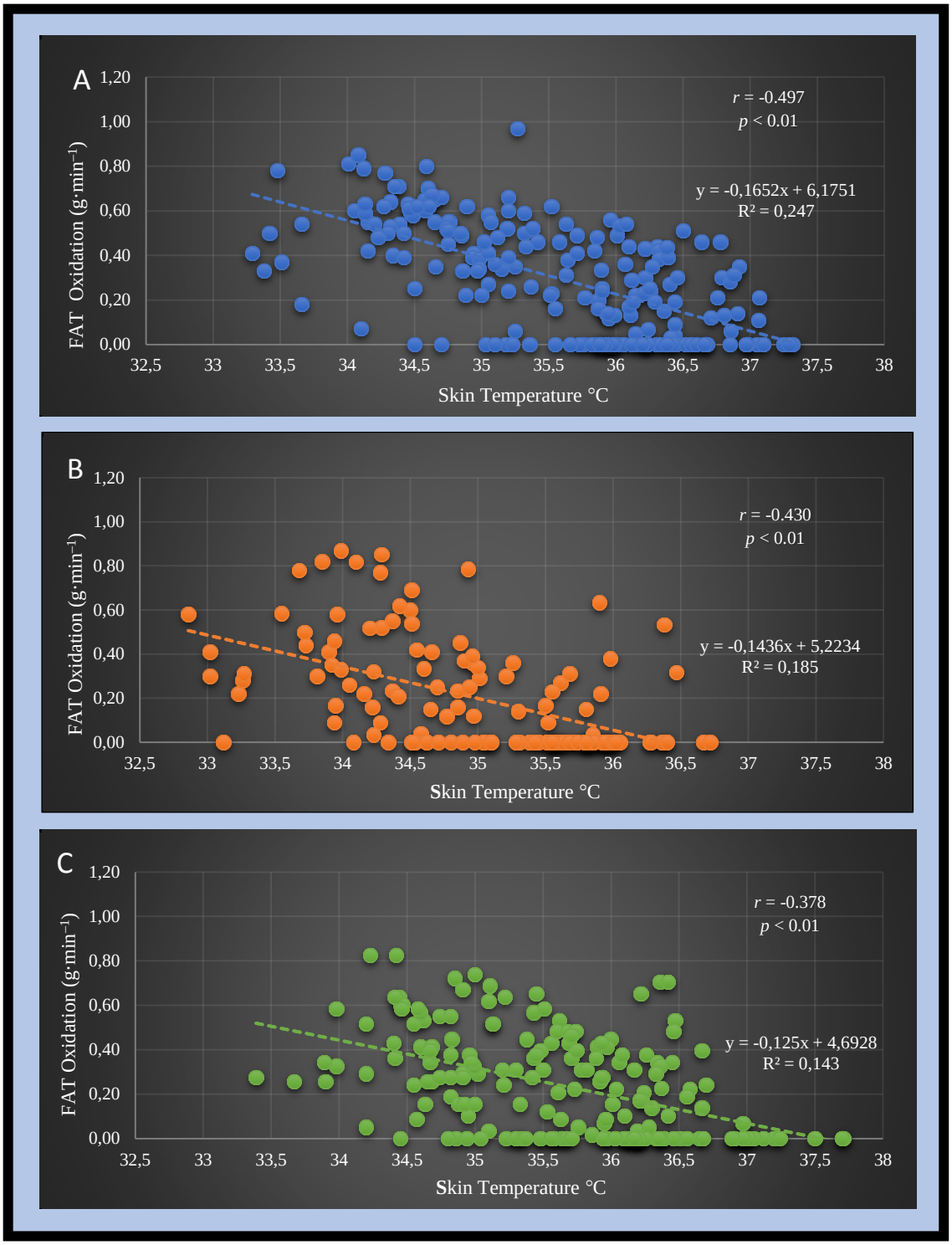

Figure 18. Relationships between FATox rates and skin temperature for all data points from **A)** high trained endurance runners, **B)** moderately active healthy runners, and **C)** professional soccer players.

Figure 19 shows the correlations between CHOox rates and Tsk for all data points points of the HT group (A); MA group (B) and PS group (C). Significant correlations were found between CHOox rates and Tsk for each group (HT: r = -0.739, p < 0.01; MA: r = 0.651, p < 0.01; PS: r = 0.726, p < 0.01).

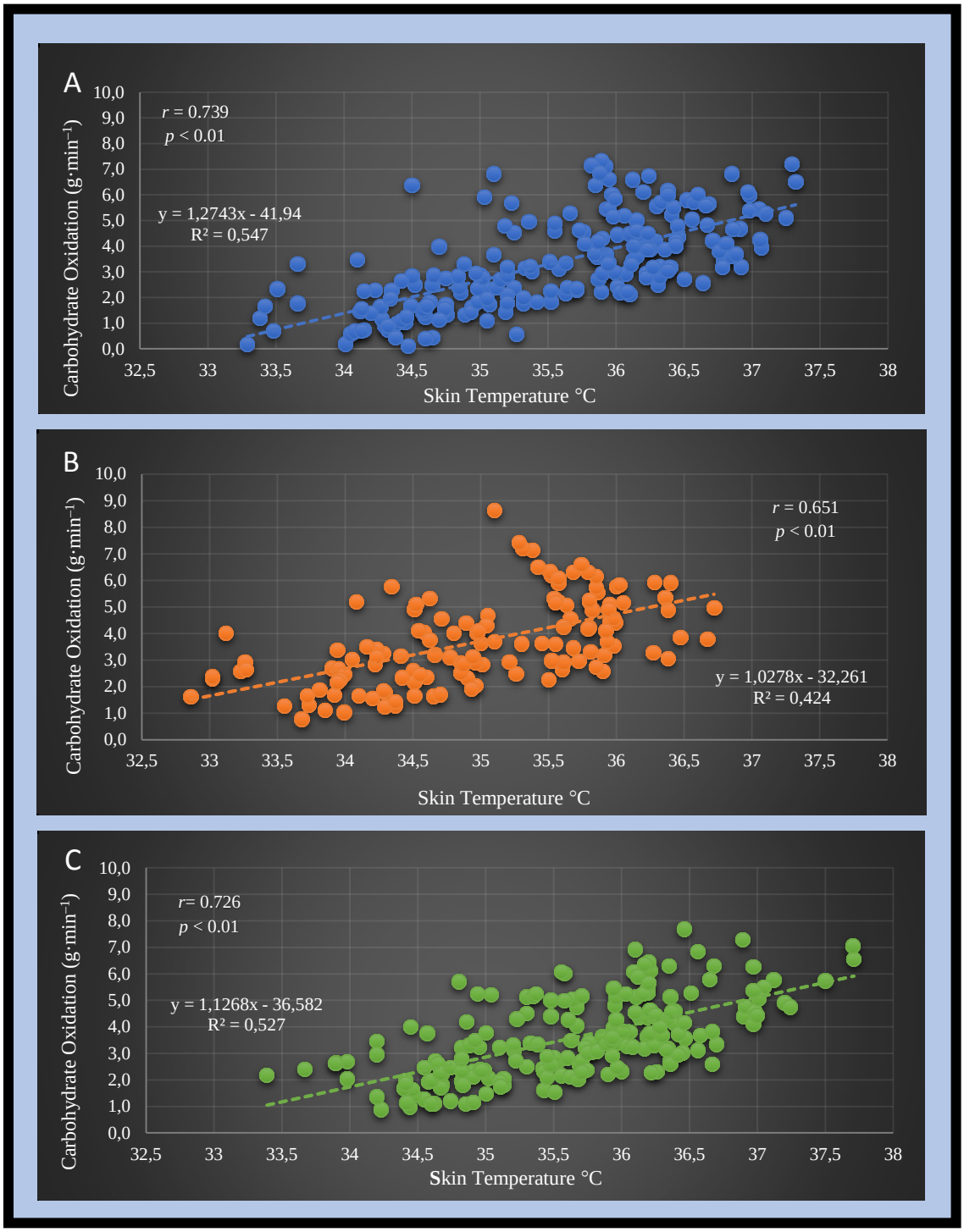

Figure 19. Relationships between CHOox and skin temperature for all data points from **A)** high trained endurance runners, **B)** moderately active healthy runners, and **C)** professional soccer players.

Table 21 shows the averages of FATox and CHOox rates, [La^{-}] concentrations, Tsk, RPE values and VO_2 at rest (baseline), during the different stages of test and during the recovery period for the HT group. In the HT group, the correlation between FATox and [La^{-}] was $r = –0.605$ ($p < 0.05$) (Table 21; Figure 20).

Table 21. Average rates of FAT and CHO oxidation, blood lactate levels, skin temperature response, RPE and oxygen uptake in an incremental maximal exercise test in high trained endurance runners.

Speed (km/h)	FATox (g·min)	CHOox (g·min)	[La^{-}] (mmol·L^{-1})	Tsk (ºC)	RPE (6-20 Borg)	VO_2 (ml/min/kg)
Baseline						
0	0.19 ± 0.05	0.10 ± 0.09	1.1 ± 0.40	34.13 ± 0.34	6 ± 0	6.48±1.74
Test						
9	0.58 ± 0.12	0.92 ± 0.47	1.1 ± 0.27	34.39 ± 0.44	8 ± 1	27.37 ± 5.45
10	0.58 ± 0.17	1.49 ± 0.54	1.1 ± 0.24	34.58 ± 0.49	9 ± 1	33.72 ± 4.18
11	0.51 ± 0.17	2.02 ± 0.42	1.2 ± 0.33	34.90 ± 0.59	10 ± 1	38.87 ± 2.62
12	0.41 ± 0.13	2.62 ± 0.42	1.3 ± 0.44	35.32 ± 0.76	11 ± 1	42.76 ± 2.67
13	0.33 ± 0.13	3.09 ± 0.38	1.8 ± 0.65	35.73 ± 0.76	13 ± 1	45.83 ± 2.45
14	0.24 ± 0.12	3.65 ± 0.53	2.6 ± 0.82	36.02 ± 0.66	14 ± 2	49.46 ± 2.53
15	0.12 ± 0.13	4.26 ± 0.57	3.6 ± 0.97	36.23 ± 0.55	15 ± 2	52.53 ± 2.67
16	0.04 ± 0.08	5.08 ± 0.70	5.0 ± 1.45	36.27 ± 0.55	17 ± 2	55.40 ± 2.36
17	0.02 ± 0.04	5.81 ± 0.72	6.0 ± 1.67	36.20 ± 0.71	19 ± 1	57.95 ± 2.13
18	0.00 ± 0.00	6.44 ± 0.83	7.0 ± 1.13	36.24 ± 0.66	20 ± 0	59.49 ± 2.18
Recovery	0.03 ± 0.03	1.00 ± 0.33	6.9 ± 1.36	36.51 ± 0.53	9 ± 1.38	10.93 ± 2.17

Note: values are means ± SD. Abbreviations: FATox (fat oxidation), CHOox (carbohydrate oxidation), [La^{-}] (blood lactate concentration), Tsk (skin temperature), RPE (rating of perceived exertion) VO_2 (oxygen uptake) and HT (high trained endurance runners) * p < .050

Relationships:

Tsk and FATox: $r = 0.187$ ($p = 0.405$)

Tsk and [La^{-}]: $r = –0.61$ ($p = 0.788$)

Tsk and CHOox: $r = –0.57$ ($p = 0.80$)

FATox and [La^{-}]: $r = –0.605$ ($p < 0.05$)

Table 22 shows the averages of FATox and CHOox rates, [La^-] concentrations, Tsk, RPE values and VO_2 at rest (baseline), during the different stages of test and during the recovery period for the MA group. The relationships between FATox and [La^-] in the MA group was $r = –0.547$ ($p < 0.05$) (Table 22, Figure 20).

Table 22. Average rates of FAT and CHO oxidation, blood lactate levels, skin temperature response, RPE and oxygen uptake in an incremental maximal exercise test in moderately active runners.

Speed (km/h)	FATox (g·min)	CHOox (g·min)	[La^-] ($mmol·L^{-1}$)	Tsk (ºC)	RPE (6-20 Borg)	VO_2 (ml/min/kg)
Baseline						
9	0.14 ± 0.06	0.17 ± 0.12	1.17 ± 0.39	33.86 ± 0.54	6 ± 0	6.04 ± 1.67
Test						
9	0.51 ± 0.23	1.68 ± 0.56	1.33 ± 0.36	33.99 ± 0.54	8 ± 1	32.44 ± 3.27
10	0.35 ± 0.24	2.49 ± 0.62	1.93 ± 0.82	34.23 ± 0.68	10 ± 2	36.32 ± 3.15
11	0.25 ± 0.19	3.09 ± 0.69	2.71 ± 1.08	34.78 ± 0.63	12 ± 1	39.62 ± 3.15
12	0.15 ± 0.18	3.63 ± 0.80	3.74 ± 1.82	35.33 ± 0.67	14 ± 2	42.49 ± 3.13
13	0.05 ± 0.10	4.50 ± 0.94	4.66 ± 1.27	35.62 ± 0.59	16 ± 2	45.72 ± 3.00
14	0.01 ± 0.03	5.31 ± 0.96	6.65 ± 1.89	35.60 ± 0.66	18 ± 2	48.26 ± 2.91
15	0.00 ± 0.00	6.01 ± 1.11	7.65 ± 1.98	35.64 ± 0.50	20 ± 1	51.26 ± 1.86
16	0.00 ± 0.00	5.93 ± 0.95	9.55 ± 0.49	35.77 ± 0.04	20 ± 0	53.56 ± 1.19
Recovery	0.01 ± 0.02	1.05 ± 0.36	7.77 ± 2	36.31 ± 0.59	9 ± 2	9.28 ± 1.39

Note: values are means ± SD. Abbreviations: FATox (fat oxidation), CHOox (carbohydrate oxidation), [La^-] (blood lactate concentration), Tsk (skin temperature), RPE (rating of perceived exertion) VO_2 (oxygen uptake) and MA (moderately active runners). * p < .050

Relationships:

Tsk and FATox: $r = 0.70$ ($p = 0.769$)

Tsk and [La^-]: $r = –0.175$ ($p = 0.459$)

Tsk and CHOox: $r = –0.108$ ($p = 0.651$)

FATox and [La^-]: $r = –0.547$ ($p < 0.05$)

Table 23 shows the averages of FATox and CHOox rates, [La^{-}] concentrations, Tsk, RPE values and VO_2 at rest (baseline), during the different stages of test and during the recovery period for the PS group. The relationships between FATox and [La^{-}] in the MA group was r = –0.547 (p < 0.05) (Table 22, Figure 20). The relationship between FATox and [La^{-}] in the SP group was r= –0.808 (p < 0.01) (Table 23; Figure 20).

Table 23. Average rates of fat and carbohydrate oxidation, blood lactate levels, skin temperature response, RPE and oxygen uptake in an incremental maximal exercise test in professional soccer players.

Speed (km/h)	FATox (g·min)	CHOox (g·min)	[La^{-}] (mmol·L^{-1})	Tsk (ºC)	RPE (6-20 Borg)	VO_2 (ml/min/kg)
Baseline						
9	0.21 ± 0.10	0.16 ± 0.16	1.1 ± 0.34	34.20 ± 0.42	6 ± 0	6.73 ± 1.82
Test						
9	0.48 ± 0.19	1.71 ± 0.58	1.2 ± 0.34	34.49 ± 0.39	8 ± 1	31.30 ± 2.82
10	0.40 ± 0.19	2.19 ± 0.55	1.5 ± 0.51	34.83 ± 0.45	10 ± 1	34.80 ± 2.27
11	0.35 ± 0.19	2.67 ± 0.69	1.9 ± 0.69	35.26 ± 0.55	12 ± 2	38.36 ± 2.48
12	0.29 ± 0.21	3.14 ± 0.74	2.4 ± 0.96	35.69 ± 0.53	13 ± 2	41.56 ± 2.22
13	0.21 ± 0.20	3.64 ± 0.82	3.1 ± 1.15	35.96 ± 0.56	15 ± 2	44.38 ± 2.41
14	0.13 ± 0.18	4.36 ± 1.00	4 ± 1.49	36.15 ± 0.64	16 ± 2	47.50 ± 2.68
15	0.07 ± 0.13	4.96 ± 1.15	5.1 ± 1.78	36.24 ± 0.70	18 ± 2	49.97 ± 2.47
16	0.05 ± 0.12	5.30 ± 1.03	5.7 ± 1.89	36.24 ± 0.62	19 ± 1	53.07 ± 3.38
17	0.02 ± 0.07	5.55 ± 0.96	6.6 ± 1.51	36.29 ± 0.73	20 ± 0	54.41 ± 3.80
Recovery	0.03 ± 0.05	0.96 ± 0.33	6.2 ± 1.59	36.32 ± 0.66	9 ± 1	10.36 ± 1.72

Values are means ± SD. Abbreviations: FATox (fat oxidation), CHOox (carbohydrate oxidation), [La^{-}] (blood lactate concentration), Tsk (skin temperature), RPE (rating of perceived exertion) VO_2 (oxygen uptake) and PS (professional soccer players). * p < .050

Relationships:

Tsk and FATox: r = 0.67 (p = 0.762)

Tsk and [La^{-}]: r = –0.090 (p = 0.683)

Tsk and CHOox: r = 0.034 (p = 0.878)

FATox and [La^{-}]: r = –0.808 (p < 0.01)

Inverse relationships were observed among the average rates of FATox and the average [La^{-}] concentrations across the three groups studied. In the HT group, the correlation between FATox and [La^{-}] was r = –0.605 (p < 0.05) (Table 20; Figure 20). While in the relationships between the averages FATox and average CHOox was only

significant in the SP group (r = –0.776 (p < 0.01). We did not find significant correlations between the average Tsk vs. average FATox or average Tsk vs. CHOox rates or average Tsk vs. [La^{-}] concentrations.

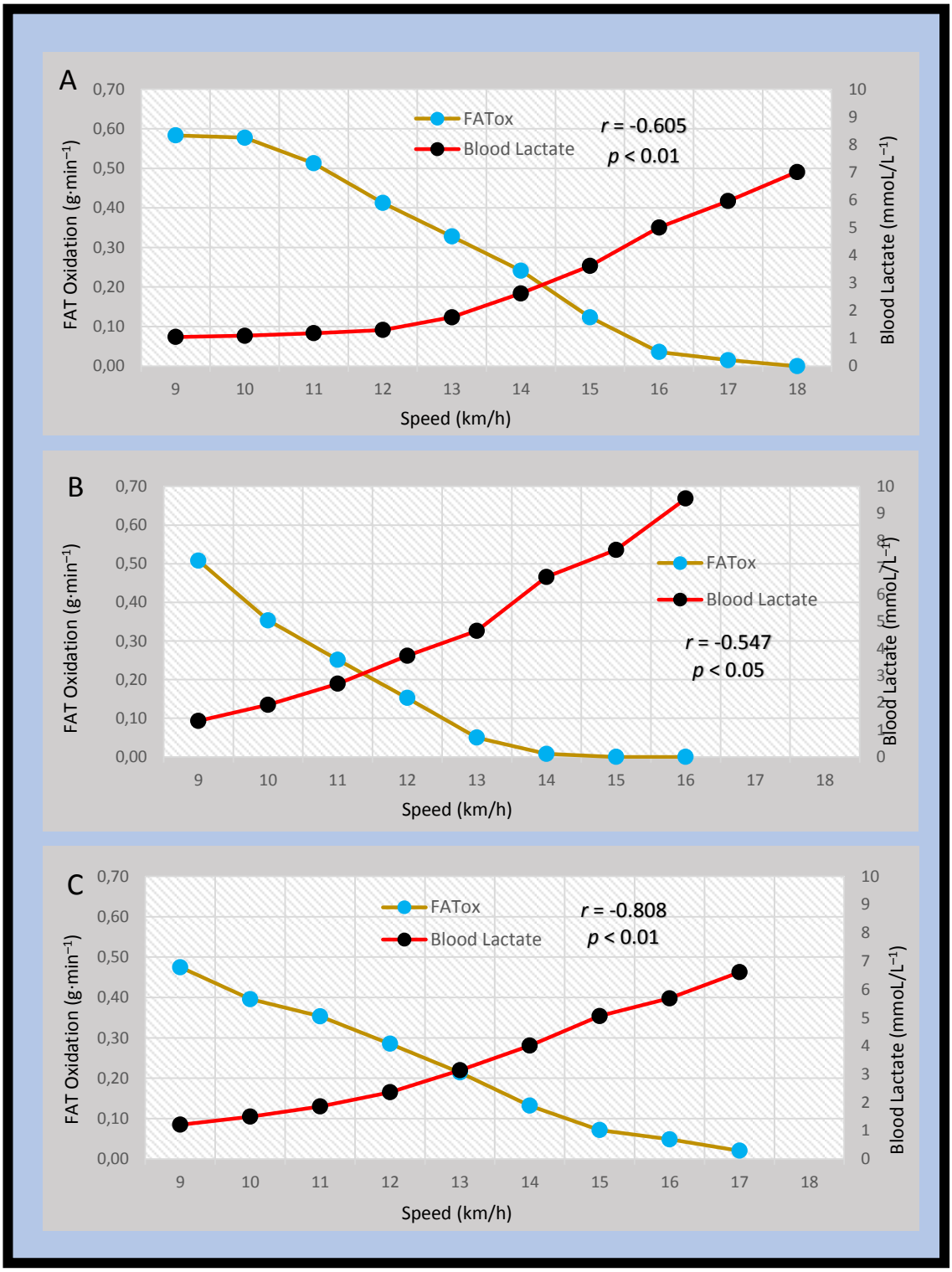

Figure 20. Relationships between the average FATox rates and blood lactate concentrations as a function of exercise speed in **A)** high trained endurance runners, **B)** moderately active healthy runners, and **C)** professional soccer players

7. DISCUSSION

7.1 First Study

In this study we hypothesized that higher aerobic capacity could be associated with an enhanced Tsk response in male endurance runners. This hypothesis was tested by comparing two groups of male endurance runners with different levels of aerobic capacity during a maximal exercise test. We observed that the HF group achieved higher Tsk values compared with the MF group, however, the Tsk_{peak} achieved by the groups were not statistically different. As shown in Figure 12, the Tsk dynamic in both groups followed a similar pattern. This may be due to the fact that all subjects were in good physical condition, based on their VO_{2peak} (Degens et al., 2019). It is unclear if the observed larger increases in Tsk for the HF group could be due to the fact that subjects with higher aerobic fitness levels may have a better skin thermoregulatory response during exercise.

The Tsk dynamic during the incremental test can be divided into three parts: (i) initial rise to Tsk_{peak}, at around 80% of workload, with an increase in Tsk in the HF and MF groups of 1.96 ºC and 1.99 ºC, respectively; (ii) a plateau of Tsk at 80 to 90% of peak workload in both groups and, (iii) a decrease in Tsk until the end of exercise. Accordingly, this pattern of increases in Tsk during exercise is consistent with previous observations in individuals with high fitness levels (Boegli et al., 2003). Our results show that as exercise intensity and VO_2 increase during the test, Tsk increases until 80-90% of peak workload is reached. It is likely that this continuous increase in Tsk is associated with active cutaneous vasodilation, resulting from increased absolute SkBF as exercise intensity increases (Etain et al., 2015). At 80 to 90% of peak workload (Figure 12), we observed a plateau of Tsk in both groups (Wong & Hollowed, 2016). From this point, both groups showed a critical and sudden decrease in Tsk, which is likely associated with decreased SkBF and cutaneous vasodilation. Based on previous studies (Takada et al., 2013), a Tsk plateau at high exercise intensities shows a withdrawal of active vasodilation, which reduces the ability of the athlete to dissipate heat, causing cutaneous vasoconstriction, which is associated with increased adrenergic activity (Okazaki et al., 2002). Our results also seem to confirm the results found by Zontak et al. (1998), in which the rate of decrement of Tsk was dependent on the intensity of the workload.

During the initial period of the recovery phase, Tsk values increased quickly in both groups due to the need to dissipate heat after exercise. In this phase, our objective was to analyze and compare the behavior and increases in Tsk in both groups. Another study also found similar patterns, and demonstrated that these increases in Tsk reflect the convective transfer of heat from the core to the periphery (Charkoudian, 2003).

Skin temperature is influenced by fitness level, as well as by other variables such as body composition, fat and muscle mass %, age, and other cardiovascular variables, like VE and HR. Aerobic fitness level, along with age, appear to be the most important limiting factors in the cutaneous vasodilation response to exercise (Kenney et al., 1997). Therefore, physical inactivity and aging contribute to reducing VO_{2peak}, which decreases heat dissipation capacity and, consequently, BT control during strenuous exercise (Reilly et al., 2006; Rowland, 2008). The MF subjects had a higher BMI and fat mass %, and lower muscle mass and VO_{2peak} (Table 14). These variables and age itself (Larose et al., 2013) have all been found to be independently and negatively associated with Tsk_{peak}, which is related to maximal cutaneous vasodilation (Boegli et al., 2003). However, future studies should evaluate VO_{2peak} and age as potential considerations in thermoregulation.

The result of the multiple linear regression shows that $Speed_{peak}$ has a significant effect on the Tsk_{peak} of participants (Table 17). Nevertheless, these results should be interpreted with caution as only 15 % of the variance in Tsk_{peak} is explained by the model.

The results of our study are similar to the results of Merla et al. (2005), in which changes in Tsk during exercise were associated with highly trained individuals, due to a major activation of the sympathetic active vasodilator system and better thermal adaptations (Pergola et al., 1996). These findings may be linked to direct and substantial vasodilation of blood vessels (Cuddy et al., 2013), which contributes importantly to SkBF, helping to reach high Tsk values. Stapleton et al. (2014) also found that higher levels of aerobic fitness were associated with an increased rate of Tsk for a given increase in mean body temperature and exercise intensity. We suggest that future

studies examine the relationship between the thermoregulatory response and the variability of HR and its contribution to the sympathetic system.

It is also unclear if higher aerobic fitness levels in the HF group may be associated with an enhanced ability to maintain higher skin thermoregulatory response (Priego Quesada et al., 2015) resulting in increased heat dissipation (Boegli et al., 2003), compared with the MF group during an incremental maximal exercise test.

During the test, changes in Tsk are accomplished primarily by blood circulation (Zontak et al., 1998), which implies that Tsk can be used as an index to predict thermal changes during exercise (H. Liu et al., 2014; Takada et al., 2013). While our results seem to indicate that Tsk response is a good indicator of training status and aerobic fitness. In future studies, it would be of interest to see if the correlation between aerobic fitness and Tsk could be a powerful predictor of aerobic performance.

We conclude that despite between-group differences in fitness level, our results did not show significant differences in Tsk increases between groups. However, we provide evidence that a moderate to high fitness level may enhance skin thermoregulation response, at least in the torso where the Tsk sensor was located. The influence of aerobic capacity shows a close relationship between Tsk and cardiovascular responses to relative exercise intensity.

7.2 Second Study

Metabolic flexibility is a term proposed well over a decade ago by Kelley and colleagues (Storlien et al., 2004). It reflects the ability to oxidize FATs and CHOs, and is associated with a good level of aerobic performance (San-Millán & Brooks, 2017). A number of studies have investigated Tsk and metabolic response to exercise (José González-Alonso et al., 1999b). In this study we hypothesized that higher metabolic flexibility could be associated with an enhanced Tsk response across populations with different metabolic characteristics. This hypothesis was tested by comparing three groups of individuals (HT competitive endurance runners, MA runners and PS players) during an incremental maximal exercise test.

We observed that the HT and PS groups achieve higher Tsk values compared with the MA group, in which the Tsk_{peak} achieved for these groups were statistically different compared with the MA group (Table 19; Figure 15). This may be due to the fact that both HT and PS groups had better physical conditioning, based on their VO_{2peak} (Degens et al., 2019; Ho et al., 1997b; Jemni et al., 2018; Selkirk & McLellan, 2001) compared with the MA group (Table 19). Regardless, although there were significant differences in VO_{2peak} (ml/min/kg) between the HT runners and the PS players (Table 19), we did not find significant differences in the Tsk_{peak} between the HT and PS groups. Furthermore, it is unclear if the observed larger increases in Tsk for the HT and PS groups could be due to the fact that subjects with higher aerobic fitness levels may have a better skin thermoregulatory response during exercise. In the course of the exercise test, the HT competitive endurance runners and PS players had a higher Tsk at any given level of workload, when compared with healthy MA subjects (Table 19). Cross-sectional data analysis have shown that endurance exercise trained individuals have greater Tsk rates, which is associated with an increased heat dissipation capacity, than sedentary individuals (Boegli et al., 2003; Ho et al., 1997a; Tankersley et al., 1991; Pierzga et al., 1999). As shown in Table 19, the relationship of Tsk_{peak} and the training status (VO_{2peak}) of individuals are positively associated during incremental exercise.

Although, soccer players have to perform at high intensities at many points throughout a game, low intensity activities are performed in more than 70% of the

game, and are primarily aerobic in nature (Jemni et al., 2018). Therefore, aerobic endurance plays a vital role in overall soccer aerobic performance (Bangsbo et al., 2007; N. Gibson, J. Currie, 2013). Taking these observations into account, the average aerobic workload is influenced by metabolic flexibility and the effectiveness of the skin thermoregulatory capacity in PS, and is also associated with aerobic capacity. As shown in Figure 15, the Tsk dynamic in both the HT and PS groups followed a similar pattern. Though PS had higher Tsk values, this might be due to the fact that PS subjects were younger, which could be associated with enhanced Tsk response during exercise (Ho et al., 1997b; Mcdonald, 2009). We recruited endurance runners, healthy active individuals and soccer players in an age range of 18-45 years, so perhaps the soccer players' age may have influenced the Tsk response. Therefore, it is unclear if higher aerobic fitness levels in the HT group may be associated with an enhanced ability to maintain a higher skin thermoregulatory response (Priego Quesada et al., 2015), resulting in increased heat dissipation (Boegli et al., 2003) compared to the PS group during an incremental maximal exercise test. Under acute stress metabolic induced thermogenesis during the test, the HT and PS groups have the ability to maintain higher increases in Tsk during a longer time compared with the MA group, which showed a drop or lower increases in Tsk. Sympathetically-mediated vasoconstriction causes a drop in Tsk (Herborn et al., 2015), and this influx of peripheral blood and adrenergic activation simultaneously increases [La^-].

Our results showed slower increases of Tsk in the MA group (Figure 13, Table 19) during the test at the same absolute submaximal exercise intensities of the HT and MA groups, which may likely be associated with decreased SkBF, cutaneous vasodilation and the formation of sweating which dissipates body heat. Skin temperature is influenced by $VO_{2peak,}$ as well as by other variables such as body composition, age and metabolic variables, FATox and CHOox rates and blood lactate concentrations. The MA subjects had a higher BM, fat mass % and VO_{2peak} compared with the HT and PS groups. Our study also shows the presence of significant differences in the relationships between Tsk with blood lactate accumulation (Figure 17) and FATox (Figure 18) and CHOox (Figure 19) rates in the three groups studied during the graded incremental exercise.

Our data show inverse relationships between FATox and Tsk across the range of exercise intensities studied in all three groups (Fig. 18). Our data also show a significantly higher capacity to oxidize FAT in the HT group, followed by the PS group and, finally, by the MA group who had a poor FATox capacity (Fig. 16). Accordingly, our data are in concordance with the scientific literature showing that endurance training increases the capacity to oxidize FFAs (Bergman et al., 1999; Phillips et al., 1996; Turcotte, Richte et al., 1992). Our data are in concordance with the scientific literature (Takada et al., 2013) that shows that an increase of Tsk, associated with increased active vasodilation, may increase the ability to oxidize FFA during exercise (Johnson et al., 2014), and allow heat to dissipate. The PS and MA groups had less ability to oxidize fat, causing cutaneous vasoconstriction, which is associated with increased adrenergic activity (Okazaki et al., 2002). Our results also seem to confirm the results found by Zontak et al. (1998), in which the rate of increases of Tsk is dependent on cardiorespiratory fitness (Ho et al., 1997b; Gibson et al., 2013; Selkirk & McLellan, 2001) and intensity of the workload (Herborn et al., 2015).

In our study, higher FATox levels and lower [La^-] at all given intensities suggest a highly developed skin thermoregulatory response in the HT and SP groups compared with the MA group. On the other hand, significantly lower FATox rates and significantly higher levels of [La^-] are associated with lower Tsk values at low exercise speed in the MA group. Therefore, both FATox rates and [La-] seem to represent a valid indirect method to test for thermoregulatory capacity and metabolic flexibility during a long bout of exercise in individuals ranging from athletes to moderately active individuals and soccer players. We observed important differences in the 'crossover' (CO) (Brooks, 1997) between all three groups (Figure 20). In the HT group, CO occurs at high absolute exercise intensities, at approximately 85% of maximal VO_{2peak}, denoting an exceptional capacity for FATox. In the MA group, CO occurs at approximately 78% of maximal VO_{2peak}, denoting a reduced capacity to oxidize FAT and an earlier reliance on CHO during the exercise test. For the SP group, CO was at approximately 82% of maximal VO_{2peak}, showing a good capacity to oxidize FAT. In conclusion, the results obtained are in concordance with San-Millán & Brooks (2017), who proposed this method of viewing FATox and [La-] during exercise (Figure 20) as

an alternative approach to assessing metabolic flexibility (Storlien et al., 2004) in clinical settings and research.

In 1962 and 1964, Issekutz et al., noted the effect of lactatemia on diminishing circulating FFA in individuals during hard exercise (Issekutz et al. 1962; Rodahl et al al., 1964). Interestingly, we can observe this phenomenon in Figure 20, which may be associated with a plateau and even sudden decrease in Tsk during hard exercise at the final stages of the test, and is also likely associated with decreased SkBF and cutaneous vasodilation. Based on previous studies (Takada et al., 2013), a Tsk plateau at high exercise intensities shows a withdrawal of active vasodilation, which reduces the ability of the athlete to dissipate heat, causing cutaneous vasoconstriction; lactate production is also greater, which is associated with increased adrenergic activity (Okazaki et al., 2002), and lactate inhibits lipolysis in fat cells through activation of a G-protein-coupled receptor (GPR81) (San-Millán & Brooks, 2017). Our results also seem to confirm the results found by Zontak et al. (1998), in which the rate of decrement of Tsk was dependent on the intensity of the workload. Not surprisingly, our data also show consistent and strong inverse correlations between [La-] and FATox in all three groups, as expected, as both are mitochondrial substrates and lactate inhibits lipolysis, as previously illustrated by other studies (San-Millán & Brooks, 2017).

As shown in Figure 20, lactatemia significantly affects and downregulates fat metabolism. Our study shows the presence of an overarching major effect of lactatemia on limiting FATox rates in individuals of widely ranging exercise capacities, as in the SP and MA groups. These findings may be explained by reduced FATox rates and increased La (lactatemia) at the same absolute submaximal exercise intensities, and decreased oxidative capacity with lower aerobic power compared with the HT endurance runners. Therefore, our results also seem to confirm the results found by (San-Millán & Brooks, 2017), in which blood lactate accumulation is negatively correlated with FATox and positively correlated with CHOox during exercise across populations with different metabolic characteristics.

Our correlations between FATox rates and [La^-] implicate differences in Tsk across the three categories of fitness level, HT > SP > MA. As previously shown, increases in FATox and lactate clearance capacity have been observed after endurance training (B. C. Bergman et al., 1999; Bryan C. Bergman et al., 1999; Messonnier et al., 2013; Phillips et al., 1996; Turcotte et al., 1992). The slope of blood lactate increases in response to exercise speed in the HT group, which was low compared with PS group who, in turn, showed lower [La^-] accumulation curve than MA subjects (Figure 16). As previously shown, increases in FATox and lactate clearance capacity in HT endurance runners have been observed in recent studies (Messonnier et al., 2013; San-Millán & Brooks, 2017). Based on other studies, we know that while [La^-] is lower in trained, compared with untrained, individuals at a given exercise PO (Bergman et al., 1999; Messonnier et al., 2013; San-Millán & Brooks, 2017), and we also know that during high-intensity exercise, lactate production, and hence [La^-] appearance rate, is greater in trained than in untrained individuals, probably as a simple mass effect of higher EE. However, [La^-] is lower due to the training adaptations in the HT and SP groups that increase [La^-] clearance capacity.

An example of remarkable lactate clearance capacity can be observed in Fig. 16, where the [La-] response in the three groups can be observed at common exercise speeds. Also remarkable is that the [La-] levels accumulated in the HT group at approximately at 13km/h are close to the [La-] observed at 10 km/h and 11 km/h in the SP group and MA group, respectively (Bergman et al., 1999; Horton et al., 1998; Messonnier et al., 2013; San-Millán & Brooks, 2017; Swart & Jennings, 2004). For both the SP and MA groups, the first increase in [La-] over baseline levels or 1^{st}-2^{nd} stages, at 9km/h and 10 km/h respectively, coincides with the decrease in FATox (Figure 20 B, C), which is possibly due to the effects of lactate on lipolysis, as described above. However, the metabolic task for the HT group is low from the start of exercise to the 13 km/h stage, at which point there are increases in [La-] concentration and a reduction in FATox levels (Figure 20 A) at the same absolute submaximal exercise intensities. In Figure 20, we can also observe that regardless of the level of aerobic capacity, all groups show that FATox is suppressed at the [La^-] level of approximately

4-6 mmol·L^{-1}. This is also possibly due to the suppression of lipolysis at high [La^{-}], and also indicates a 'threshold-like' phenomenon in all subject groups.

We conclude that despite between group differences in fitness level, age and oxidative capacity, our results show significant differences in Tsk increases, FATox rates and [La^{-}] concentrations between all groups. However, we provide evidence that a moderate to high fitness level may enhance the skin thermoregulation response, at least in the torso where the Tsk sensor was located. The influence aerobic capacity has shows a close relationship between Tsk and metabolic flexibility relative to exercise intensity. These differences should be taken into account in the training and nutritional strategies for enhancing professional soccer and endurance runner performance.

7.3 Both Studies

Based both on previous studies and on our findings, it is clear that Tsk response plays an important role in the thermoregulatory process during maximal exercise (Cuddy et al., 2013; Pierzga et al., 2003; Schlader, Simmons, Stannard, & Mündel, 2011a; Thomas et al., 1999). We examined Tsk response using a maximal test between two types of experimental protocols with different stages, however we didn't examine the effects of a submaximal or graded experimental protocol on Tsk response, and the relationship with other physiological variables in other populations. We hypothesized that the use of longer stages may be helpful to elicit a proper 'steady state' status, and are therefore quite useful for gathering accurate thermoregulatory and metabolic data. It could be possible that the duration of the test, according to our protocol, may not be enough to register adaptations in other populations with less training. We provide further insight into the methodological considerations for future studies in this area.

Despite the fact that Tsk is an important measurement and contributor to thermoregulation and aerobic performance, it should be taken into account that Tsk is mainly a consequence of other important factors such as Tc, SkBF and the environmental temperature (Xu et al., 2013). For future studies, it would be of interest

to analyze if this correlation between aerobic fitness and Tsk may be strongly affected by these factors and other parameters.

Regarding Tc, it was not measured during the incremental test. Core temperature could show that differences in body temperature are due to differences in fitness level, as reported by the literature (Okazaki et al., 2002; Stapleton et al., 2014; Wong & Hollowed, 2016). The relationship of this thermal behavior with Tc and metabolic responses could help to better determine thermoregulatory response during exercise. Hence, it would be of interest to study the relationship between skin thermal behavior related to Tc and other metabolic responses during incremental exercise with longer stages in different populations.

8. CONCLUSIONS

8.1 General Conclusions

The aims of this project was divided into two studies:

1. A first study analyzed and compared the correlation between Tsk and cardiorespiratory variables during an incremental maximal stress test in high fit (HF) and moderately (MF) male endurance runners.

2. A second study analyzed and compared the correlation between Tsk and metabolic flexibility by measuring [La^-] along with FATox and CHOox rates during an incremental maximal stress test in highly trained (HT) competitive endurance runners and moderately active (MA) male runners, and professional soccer (PS) players.

Through these studies, we propose:

1. To analyze the dynamic of Tsk response in different populations during an incremental exercise test to VO_{2peak} on an ergometer treadmill.
   These findings indicated that VO_{2peak} was positively associated with the increased Tsk during incremental exercise in male endurance runners.

2. To analyze the recovery period of five minutes after the same exercise tests, and the changes that could appear in both studies.
   In this phase, our objective was to analyze and compare the behavior and increases in Tsk in different groups. These results in both studies showed higher and faster increases in Tsk in trained subjects after the exercise. Higher VO_{2peak}, taking into account the age of the PS group, which is associated with an increased skin thermoregulatory capacity. These increases reflect a major convective transfer of heat from the core to the periphery, as heat dissipation.

3. To determine the skin thermoregulatory response related to thermal changes during exercise. Hence, Tsk measurements could offer indirect hemodynamic information of vasodilation response during exercise-related thermal adjustment.

   In the first study, Tsk_{peak} correlated with VO_{2peak}, $Speed_{peak}$, HR and VE, which showed the association between hemodynamic changes and thermoregulatory responses. The dissipation of heat from the deeper parts to the skin is accomplished primarily by blood circulation, so we can use the Tsk as index to predict thermal changes during exercise.

4. To determine if the validated exercise protocol of Achten (2003) can provide a measurement of maximal Fat oxidation (MFO) rate and assess the correlation with thermoregulatory response.

   We used two types of maximal test, one with stages of 2 minutes and other with 3 minute stages, to determine over a wide range of exercise intensities, with the objective to found small intraindividual variation. Based on other studies, the use of longer stages may be helpful to elicit a proper 'steady state' status and the metabolic flexibility and thermoregulatory response. We did not analyze this.

5. To determine the correlation of $VO_{2peak,}$, $[La^-]$ concentrations, FATox and CHOox rates, skin thermoregulatory response, RPE values, Fat % and Mass muscle % in HT endurance runners, MA runners and professional soccer players.

   These results showed that the HT group exhibited significantly higher Tsk values, $VO_{2peak,}$ FATox rates and lower $[La^-]$ concentrations, RPE values at the same absolute submaximal exercise intensities, BMI, fat mass% compared with MA group. Compared with PS group, who showed significant differences in age, also showed significant differences with higher Tsk values, FATox rates and $speed_{peak}$ than MA group during the test.

8.2 Hypothesis

First study

1. We hypothesized that higher aerobic capacity could be associated with an enhanced response of Tsk in male endurance runners.
   The main findings of the present study indicate that VO_{2peak} was positively associated with increased Tsk during incremental exercise in male endurance runners.

2. We hypothesized that HF endurance runners have the ability to maintain higher Tsk response than MF runners during maximal exercise.
   Results revealed that the MF group exhibited lower Tsk at baseline, and at 60% and 70% of peak workload compared with HF group. However, our results do not show that a higher level of aerobic fitness in male HF contributes to achieve higher Tsk, or a greater thermoregulatory capacity to dissipate heat during exercise.

3. We hypothesized that Tsk measurement can provide indirect hemodynamic information of vasodilation response during exercise-related thermal adjustment and can be used as a predictable index of vasodilatation response. These results showed that Tsk_{peak} correlated with VO_{2peak}, $Speed_{peak}$, HR and VE in both groups, resulting that Tsk dynamic may reflect and may serve as a tool for assessing the integrity of these thermoregulatory mechanisms as part of the circulatory system, which interacts with the thermal and hemodynamic responses. These findings also may reflect a better understanding of the sympathetic vasodilator system and thermal adaptations.

4. We hypothesized that Tsk can be used as an index to predict aerobic fitness.
   Our results did not show that a higher level of aerobic fitness in male endurance runners contributes to achieving higher Tsk during exercise, but it

reflects the relationships between other cardiorespiratory and metabolic responses during exercise in different groups.

Second study

1. We hypothesized that higher metabolic flexibility could be associated with an enhanced response of Tsk in well-trained endurance and active runners and professional soccer players.

   We observed that the HT group and PS groups achieved higher Tsk values compared with the MA group, in which the Tsk_{peak} achieved for both of these groups were statistically different compared with the MA group. This may be due to the fact that both HT and PS groups had better physical conditioning. Results showed a poor metabolic flexibility in MA group, associated a decreased to oxidize fat and higher lactate concentrations during the test. Our study also showed the presence of significant differences in the relationships between Tsk with blood lactate accumulation and FATox and CHOox rates in the three groups studied during the graded incremental exercise.

2. We hypothesized that HT endurance runners have the ability to enable a higher Tsk rates than MA runners and SP players during absolute submaximal exercise intensities.

   We observed that the HT group and PS groups both achieved statistically higher Tsk values compared with the MA group.

3. We hypothesized that HT endurance runners have higher metabolic flexibility, therefore a higher oxidative and lactate clearance capacities.

   These findings showed a decreased FATox rates and increased La (lactatemia) at the same absolute submaximal exercise intensities in MA compared with HT and PS groups. This decreased oxidative capacity with lower aerobic power compared with the HT endurance runners, seem to confirm the results of other studies, in which blood lactate accumulation is negatively correlated with FATox and positively correlated with CHOox

during exercise across populations with different metabolic characteristics. Therefore, poor lactate clearance capacity due to low metabolic flexibility affects skin thermoregulation, which could result in thermoregulatory dysregulation, resulting in a decreased ability to dissipate heat during exercise.

8.3 Limitations

This study has some limitations, such as not using longer stages during the test to determine changes in Tsk and metabolic responses. It is important to acknowledge the limitations of this study. Firstly, between-group difference on Tskpeak, which was ~0.3ºC, should be interpreted with caution as the accuracy of the device assessing the Tsk is ± 0.2 °C. Nevertheless, all participants were assessed by using the same device and methodology, so all assessments may present the same measurement error. Furthermore, in order to reduce the influence of sweat evaporation, the Tsk sensor was placed in a location where evaporative heat loss was minimal (Xu et al., 2013). In this study, the sensor on the torso was covered by elastic adhesive cloth. The low permeability of the cloth reduces evaporative heat loss in the covered area and, thus, likely reduces the error and increases the accuracy of the Tsk measurement.

Secondly, this is a cross-sectional study, and did not study the effects of specific endurance training programs, so differences between groups may be due to differing physiological capacities to control the skin thermoregulatory response during exercise. Thirdly, Tc was not measured during the incremental test. Core temperature could show that differences in body temperature are due to differences in fitness level, as reported by the literature (Etain et al., 2015; Stapleton et al., 2014; Wong & Hollowed, 2016). The relationship of thermal behavior with Tc and other metabolic responses could help to better determine thermoregulatory response during exercise.

Hence, it would be of interest to study the relationship between skin thermal behavior related to Tc and other metabolic responses during graded and progressive exercise in different populations. Finally, sweat on the skin surface at the end of exercise could have influenced Tsk data and should also be considered a limitation of

the present study. Ammer (2009) suggested that a film of water on the skin may work as a filter and, therefore, could lead to an underestimation of thermal data.

Moreover, it could be possible that the duration of the test, according to our protocol, may not be sufficient for registering adaptations in other populations, such as untrained individuals.

The participants in this study had a range of aerobic fitness levels and participate in different endurance sports activities, such as distance running, mountain running, triathlon and soccer. This is a strength when it comes to ensuring that the results of the present study not only apply to runners with high aerobic fitness, but also to those exercising at a high recreational level. Future studies should study the skin thermoregulatory response in female endurance runners, and compare the results with those of their male counterparts. Also, it would be interesting to compare and analyze the difference between peripheral and core temperatures of male and female runners. Our study does not discriminate between subjects in each group, especially between the HT and MA groups.

Further limitations of our study reside in the indirect nature of the assessment of metabolic flexibility and skin thermoregulatory response. Although we obtained robust data from indirect parameters of metabolic flexibility and thermoregulatory response, the ideal would be to directly study, through Tc measurements and other methods such as muscle biopsies or tracers, the ability to oxidize both FFA and [La^-].

Another limitation of our study that should be highlighted is that this is a cross-sectional study and did not study the effects of specific endurance training programs, where the differences between both groups may be due to different physiological capacity to control the skin thermoregulatory response during exercise. Regarding to Tc was not measured during the incremental test. Core temperature could show that differences in body temperature are due to differences in fitness level, as reported by the literature (Etain et al., 2015; Stapleton et al., 2014; Wong & Hollowed, 2016). The relationship of this thermal behavior with the Tc and other metabolic responses could

help to better determine thermoregulatory response during exercise. Hence, it would be of interest to study the relationship of skin thermal behavior related to Tc and other metabolic responses during graded and progressive exercise in different populations. Finally, sweat on the skin surface at the end of exercise could have influenced Tsk data and should also be considered a limitation of the present study. Ammer (2009) suggested that a film of water on the skin may work as a filter and, therefore, could lead to an underestimation of thermal data.

8.4 Future Directions for Research

In future studies, it would be of interest to see if the correlation between aerobic fitness and Tsk is a powerful predictor of aerobic performance. However, future studies should also evaluate the influence of VO_{2peak} and age on thermoregulation.

The relationship between Tsk response and Tc and other metabolic responses could help to better determine thermoregulatory response during exercise. Hence, it would be of interest to study the relationship of skin thermal behavior related to Tc and other metabolic responses during graded and progressive exercise in different populations. Also, it would be of interest to evaluate the correlation of Tc and metabolic flexibility, which may be powerful predictors of aerobic performance.

Future studies are needed to determine the accuracy of analyzing Tsk response in different areas of the body during varying intensities of exercise. It may help to obtain more accurate values when skin thermoregulatory response and other physiological responses are evaluated.

Future studies should study the skin thermoregulatory response in female endurance runners, and compare the results with those of their male counterparts. Also, it would be interesting to compare and analyze the difference between peripheral and core temperature of male and female runners.

For future studies, it would be of interest to evaluate the correlation of Tc and metabolic flexibility, which may be powerful predictors of aerobic performance.

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10. APPENDIX

10.1 Appendix I- Test Protocols

Protocolo de pruebas

DESCRIPCIÓN DE LA REVISIÓN MEDICO DEPORTIVA AVANZADA

Se recomienda leer atentamente todas las siguientes indicaciones para realizar un RMD totalmente completo.

Documentos a aportar el día de la prueba(opcional): análisis de sangre -orina recientes, informes médicos de cualquier patología o proceso que está siendo seguido por un especialista, informes de pruebas de esfuerzo previas.

El **RMD** cuenta con diferentes partes:

- **Anamnesis completa**: Se trata de hacer un historial médico – deportivo (antecedentes médicos, deportivos, historia clínica-deportiva, etc)
- **Exploración funcional del aparato locomotor**: exploración motriz para observar posibles dismetrías y problemas osteoarticulares para prevenir riesgos de lesiones y optimizar el rendimiento.
- **Análisis antropométrico**: Conjunto de medidas encaminadas a la valoración de porcentajes de grasa, muscular, óseo, además de observar posibles dismetrías.
- **Espirometría**: Valoración de la capacidad pulmonar en reposo

Test Ergoespirométrico o Prueba de Esfuerzo:

Consiste en la realización de una prueba progresiva en máxima en tapiz rodante (cinta ergométrica).

- **Cinta ergométrica**: El protocolo se adaptará dependiendo de la disciplina deportiva y capacidad funcional y objetivo del estudio.

Es importante tener en cuenta que la mayoría de los investigadores han observado que el VO_2 Max es aproximadamente un 10 % más elevado cuando se evalúa en un tapiz rodante en comparación con la bicicleta ergométrica (American Collage Of. Sports Medicine, 2000).

La prueba se realiza con monitorización cardioventilatoria continua. Las variables ventilatorias y metabólicas recogidas son las siguientes:

- **Consumo de oxígeno, respuesta metabólica** (uso de los diferentes sustratos energéticos), Volumen ventilatorio, volumen corriente, consumo de oxígeno, Pulso de oxígeno, producción de dióxido de carbono, cociente respiratorio, equivalentes respiratorios para el O_2 y el CO_2…
- **Monitorización electrecardiográfica y TA** (al inicio, calentamiento y final de la prueba de esfuerzo).
- **Otros parámetros** como: Temperatura corporal, saturación y lactacto (muestra sanguínea que se obtiene del lóbulo de la oreja)
- Interiorizar el **Test de Borg:** para valorar la percepción de fatiga (adjuntamos explicación para que os familiaricéis con esta escala para utilizarla el día del test)

Valoración de la percepción de esfuerzo (Escala de Borg)

Las valoraciones en la percepción del esfuerzo nos ayudan a comprender mejor el trabajo realizado. Estas valoraciones son complementos importantes en las medidas fisiológicas y funcionales del rendimiento físico y en la capacidad de trabajo (Borg GA, 1982).

En los estudios sobre trabajo y rendimiento es importante evaluar varios síntomas subjetivos, quejas y molestias. Para medir estos síntomas, se pueden utilizar las escalas de percepción psicofísicas. Los aumentos en el ritmo de trabajo están asociados con valoraciones más altas en las escalas de percepción. En el campo de trabajo físico intenso y en la percepción de esfuerzo, uno de los métodos más populares es la utilización de la escala proporcional de categorías de percepción de esfuerzo.

6	Reposo o no se siente nada
7	Extremadamente suave
8	
9	Muy suave
10	
11	Suave
12	
13	Ligeramente Fuerte
14	
15	Fuerte
16	
17	Muy Fuerte
18	
19	Muy, muy Fuerte
20	Esfuerzo máximo

IMPORTANTE TENER EN CUENTA

Pautas recomendables para llegar en buenas condiciones al RMD

- *El día antes intentar descansar y preparar la prueba como si fuera un día previo a una competición*. Se trata de preparar una prueba máxima y llegar en las mejores condiciones.
- *Desayunar con total normalidad, al menos 2h/1h30' antes del RMD*.
- *Hidratación en todo momento, después del desayuno y antes de la prueba*. Llegar en fase de deshidratación marcara una prueba no válida, debido al momento a la aparición temprana de la fatiga.
- Duración del RMD, aproximadamente, de *1h30' -2h de tiempo*.
- Venir con vestimenta adecuada para una prueba de esfuerzo (**ropa deportiva**, en caso de practicar esta modalidad deportiva):
    - **Cinta ergómetrica:** venir en las mejores condiciones de carrera, *simular la vestimenta de carrera* (zapatillas de competición)

10.2 Appendix II- Informed Consent

CONSENTIMIENTO INFORMADO

REGLAMENTO EUROPEO DE PROTECCION DE DATOS

Identificación del Responsable: Le informamos que los datos personales que Usted nos proporciona son incorporados a un tratamiento de datos personales denominado PACIENTES cuyo responsable es ANDREA SUAREZ SEGADE con CIF 44810725D y con domicilio en CL. NORTE, 74, 1º-1ª, 08950 de ESPLUGAS DE LLOBREGAT, BARCELONA (ESPAÑA). Puede contactar con el Responsable, bien por teléfono en el número 606621768 o bien mediante correo electrónico en el buzón info@labsportsalud.com

Delegado de Protección de Datos: No hay Delegado de Protección de Datos Designado
Finalidad: La finalidad es la prestación de servicios médicos, concretamente el ámbito deportivo (revisiones federativas) y, realización y diagnóstico de pruebas de esfuerzo.
Plazo de Conservación: El plazo de conservación será una vez finalizada la prestación de los servicios médicos, el estipulado por la legislación vigente en materia Sanitaria y de Historia clínica.

Decisiones automatizadas y elaboración de perfiles: No se toman decisiones automatizadas ni se elaboran perfiles.

Base Jurídica del Tratamiento: La base jurídica del tratamiento es la prestación de un servicio médico.

Destinatarios de cesiones: No se prevén realizar cesiones, salvo aquellas que están autorizadas por ley.

Transferencias Internacionales: No se realizan transferencias internacionales.

Derechos: De acuerdo con la legislación vigente tiene los siguientes derechos:
- Derecho a solicitar el acceso a sus datos personales.
- Derecho a solicitar su rectificación o supresión.
- Derecho a solicitar la limitación de su tratamiento
- Derecho a oponerse al tratamiento.
- Derecho a la portabilidad de los datos.

Para ejercer sus derechos, debe dirigirse al responsable, solicitando el correspondiente formulario para el ejercicio del derecho elegido. Opcionalmente, puede redirigir al interesado a la Autoridad de Control competente para obtener información adicional acerca de sus derechos.

Por todo lo referido, manifiesto que he estado informado que todos los datos de carácter personal, incluidos los de salud, que me requieran serán objeto de tratamiento con la finalidad de gestionar y prestar los servicios sanitarios y médicos que he solicitado o solicitaré, y con tal fin, ANDREA SUAREZ SEGADE, dispone de ficheros automatizados y manuales donde quedan registrados los datos con las finalidades indicadas, por lo que:

CONSENTIMIENTO: En Cornellá a ..... del ..... Dn./a, ... con DNI / NIE ..... en nombre propio, o como representante legal de Dn/a, ……. con DNI/NIE ……., doy mi consentimiento expreso de acuerdo con lo expuesto en el presente documento.

FIRMA

Nota: en caso de menores de catorce años será necesaria la autorización de su representante legal.

10.3 Appendix III- Participant Information

Hoja de información para el participante

Título del proyecto: "*Termorregulación en adultos que entrenan deportes de resistencia"*

Estimado participante,
Le agradecemos el interés demostrado en el estudio "**Termorregulación en adultos que entrenan deportes de resistencia".**

Objetivo del estudio: Evaluar la respuesta termoregulatoria en adultos entrenados en deportes de resistencia durante una prueba progresiva de esfuerzo máxima en tapiz rodante (cinta ergométrica), junto con la valoración de otras variables cardiorespiratorias y metabólicas. Estos parámetros serán evaluados y comparados entre diferentes grupos de sujetos con diferentes niveles de condición física.

Participación voluntaria: Su participación es totalmente voluntaria. El participante es libre de aceptar ser incluido o no en el estudio. Esta decisión no afectará en ningún caso su atención o relación con los profesionales del centro ni con los profesionales que forman parte del equipo investigador del presente estudio. En caso de que haya discrepancia en la decisión de participar o no en el estudio entre el participante y el profesional, se descartará la participación del propio participante.

Procedimientos del estudio: Se solicitará la colaboración en el estudio a personas adultas mayores de 18 años que cumplan con los requerimientos en la práctica de la actividad física en cualquier disciplina en deportes de resistencia, haciendo ejercicio físico al menos 3 veces a la semana y un mínimo de 150 minutos a la semana. Además de ser capaces de correr sin limitaciones físicas importantes y sin alteraciones que puedan contraindicar su participación en pruebas de esfuerzo máximo y programas de actividad física.
Se seguirá un protocolo diseñado por un equipo de investigadores que ha sido evaluado y aprobado por el Comité Científico y Ético de la facultad de la Blanquerna. Se realizarán pruebas progresivas de esfuerzo máximo con diferentes protocolos e intensidad, según el nivel y la condición física. La inclusión en uno u otro programa se determinará al azar, sin poder escoger en qué grupo participar. Las pruebas y protocolos han sido diseñadas específicamente para personas con diferentes niveles de condición física, y se tendrá en cuenta las características individuales y el deporte de resistencia que practique. Adicionalmente, se podrán realizar sesiones de seguimiento individual para observar los cambios en la condición física de cada participante.

Durante la **visita inicial al laboratorio**, además de explicarle el proyecto y de entregarle el consentimiento informado para ser firmado por el participante, se lo citará un día en el cual se realizará una primera parte de anamnesis completa que incluirá el historial médico – deportivo (antecedentes médicos, deportivos, historia clínica-deportiva, etc.). Todo con el objetivo de evaluar el estado de salud y los niveles de actividad física. Durante **una segunda visita**, evaluaremos el resto de pruebas laboratorio (exploración funcional del aparato locomotor, análisis antropométrico para valorar

composición corporal, la fuerza, y evaluaremos parámetros de la función cardiovascular en reposo y su capacidad aeróbica mediante una prueba de esfuerzo máxima y progresiva. Durante la prueba se trata de un protocolo incremental llegando a niveles máximos de intensidad y de esfuerzo.

Durante o después de las evaluaciones de la condición física es posible que el participante sienta cansancio o alguna molestia muscular. Asimismo, los ejercicios a realizar durante el programa podrían provocar algún tipo de molestia muscular y/o fatiga, pero estas molestias desaparecerán con el transcurso del programa. En el caso de que el participante sufriera alguna molestia, dolor o problemas durante las evaluaciones o en el transcurso del estudio, tendrá la posibilidad de hablarlo el mismo día con el investigador principal o con los investigadores encargados, los cuales le brindarán la posibilidad de una visita suplementaria y si el participante acepta, se informará al médico de cabecera del participante para que pueda realizar un seguimiento en caso de lesión o enfermedad.

Luego de ambas visitas y de finalizadas las evaluaciones, le informaremos en qué tipo de intervención será incluido. Una vez realizadas las evaluaciones iniciales y el protocolo, cada participante será citado nuevamente después de dichas evaluaciones para evaluar posibles cambios en la condición física.

Confidencialidad: Todos los datos recogidos serán tratados de manera confidencial. En las listas de trabajo del estudio sólo constará el código asignado a cada participante del estudio. En el informe final o en caso de comunicarse los resultados de este estudio a la comunidad científica, la identidad del participante permanecerá totalmente anónima.

De conformidad con la Ley orgánica 15/1999, de 13 de desembre, de protección de datos de carácter personal, y la normativa que la desarrolla, le informamos que los datos personales facilitados en este formulario pasarán a formar parte de un fichero informático que estará bajo la responsabilidad de la Facultad de Psicologia, Ciencias de la Educación i del Deporte de Blanquerna - Universidad Ramon Llull y Labsportsalud, al cual solamente tendrá acceso el Investigador Principal del proyecto (Jonathan Galán Carracedo). Estos ficheros serán guardados para poder explotar los datos en futuros proyectos de investigación relacionados con los deportes de resistencia y este tipo de poblaciones, teniendo en cuenta que la termorregulación y la edad forma parte de este estudio, y el interés que significa poder realizar un seguimiento a largo término. Así y todo, podrá ejercer sus derechos de acceso, rectificación, cancelación y oposición por escrito, mediante correo electrónico, fax o correo postal a: jonathangc@blanquerna.url.edu, o bien mediante una carta dirigida a la Secretaria de la FPCEE Blanquerna (C/ Císter 34, 08022 Barcelona) mediante un escrito con fotocopia de su DNI. En tal caso, el fichero no será almacenado y será destruido una vez se acabe el proyecto actual.

Responsabilidad del estudio: El equipo investigador asume la responsabilidad del estudio. Si desea realizar alguna pregunta o aclarar algún tema relacionado con el estudio o si precisa ayuda para cualquier problema de salud relacionado con el estudio, por favor, no dude en ponerse en contacto con:

Facultat de Psicologia, Ciències de l'Educació i de l'Esport
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Laboratorio de Fisiología del Ejercicio - Labsportsalud
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08940 Cornellà (Barcelona)

10.4 Appendix IV – Colaborations Letters

Blanquerna UNIVERSITAT RAMON LLULL | Facultat de Psicologia, Ciències de l'Educació i de l'Esport

Carta de solicitud de colaboración para el centro

A la atención de la Directora Andrea Suarez Segade y Adjuntos del centro y laboratorio……

Estimados Señores,

Nos es grato dirigirnos a la Dra. Andrea Suárez y al resto de integrantes del laboratorio para expresar nuestros cordiales saludos e informarles sobre el proyecto titulado "**Termorregulación en adultos que entrenan deportes de resistencia**", cuyo objetivo es la evaluación de la respuesta termorreguladora, junto con otras variables cardiorrespiratorias y metabólicas, en diferentes sujetos entrenados en deportes de resistencia en personas mayores de 18 años con diferentes niveles de condición física.

El presente estudio también cuenta con la colaboración de investigadores de Estados Unidos y otros colaboradores de la Universitat Ramon Llull de Barcelona.

La entidad coordinadora es la Facultat de Psicologia, Ciències de l'Educació i de l'Esport Blanquerna (Universitat Ramon Llull) y además es la responsable de llevar a cabo las distintas evaluaciones e implementación de los protocolos de las pruebas.

Mediante la presente, quisiéramos solicitarles vuestra colaboración a la hora de llevar a cabo las pruebas en el laboratorio y a la hora reclutar participantes. Hemos estimado que necesitaríamos evaluar entre 70-100 sujetos que practiquen deportes de resistencia con una edad a partir de 18 años. Dichos participantes deberán presentar nivel de condición física óptimo para rendir durante pruebas incrementales y máxima de esfuerzo, y tratarse de participantes que tengo una práctica habitual de actividad física en deportes de resistencia, al menos 3 veces a la semana y un mínimo de 150 minutos a la semana.

Si están interesados en participar, les agradeceríamos que nos hicieran llegar un correo electrónico respondiendo a la presente carta. De esta manera podremos concertar un día y hora para realizar una reunión en vuestro laboratorio y les informaremos con más detalles del estudio (objetivos, reclutamiento de participantes, tests/protocolos a realizar y duración de las pruebas), así como cualquier otra información que puedan necesitar.

Desde ya les agradecemos vuestra colaboración y les pedimos disculpas por cualquier molestia ocasionada.

Atentamente,

Jonathan Galan Carracedo
Facultat de Psicologia, Ciències de l'Educació i de l'Esport
Blanquerna (Universitat Ramon Llull)
C/ Císter 34, 08022 Barcelona
+ 34677869387 email: jonathangc@blanquerna.url.edu

10.5 Appendix V- Authorization for Images Publication

Blanquerna UNIVERSITAT RAMON LLULL | Facultat de Psicologia, Ciències de l'Educació i de l'Esport

LABSPORTSALUD FISIOLOGIA DEL EJERCICIO Y VALORACIÓN DE RENDIMIENTO

Hoja autorización para la publicación de imágenes

Con la inclusión de las nuevas tecnologías y la posibilidad de que en estos puedan aparecer imágenes suyas durante la realización de las actividades de medicina deportiva. Y dado que el derecho a la propia imagen está reconocido el **artículo 18. De la Constitución** y regulado por la **Ley 1/1982, de 5 Mayo**, sobre el derecho al honor, a la intimidad personal y familiar y a la propia imagen y la **Ley 15/1999, 13 de Diciembre**, sobre la Protección de datos de Carácter Personal. La dirección de este centro y estudio pide la autorización para poder publicar las imágenes en las cuales aparezca individualmente o en grupo, en las diferentes secuencias y actividades realizadas en nuestro centro.

CONSENTIMIENTO: En ..... a ..... Dn./a, ... con DNI / NIE ..... doy mi consentimiento expreso de acuerdo con lo expuesto en el presente documento.

Jonathan Galán Carracedo
Facultat de Psicologia, Ciències de l'Educació i de l'Esport
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10.6 Appendix VI- Medical Screening

NOMBRE Y APELLIDOS: _____

D.N.I: ***EDAD:*** ***FECHA NACIMIENTO:***

CLUB: ***TELF.*** ***E MAIL:***

ANTECEDENTES FAMILIARES

¿Algún miembro de su familia directa ha padecido o padece algunas de las siguientes enfermedades? Indique *a la derecha su parentesco (padre, madre, hermano/a, abuelo, tío/a)*

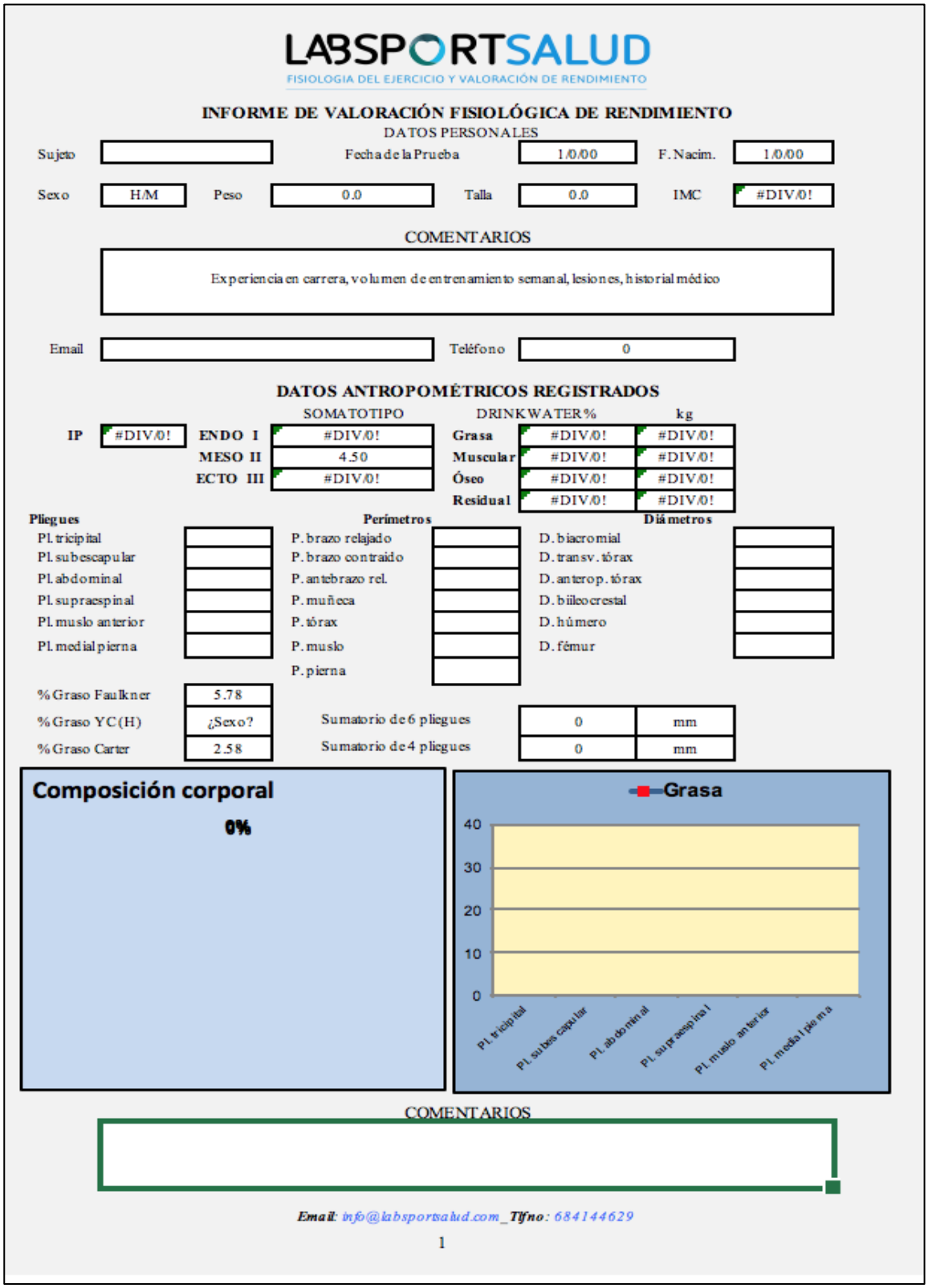
- ☐ Antecedentes de muerte súbita
- ☐ Diabetes
- ☐ Enfermedad cardiaca (angina de pecho, infarto de miocardio, congénita)
- ☐ Accidente cerebro-vascular (derrame, embolia, infarto cerebral)
- ☐ Hipertensión arterial
- ☐ Obesidad
- ☐ Colesterol elevado
- ☐ Tuberculosis
- ☐ Alcoholismo
- ☐ Enfermedades mentales
- ☐ Cáncer
- ☐ Alergias, asma
- ☐ Anemias u otras enfermedades de la sangre
- ☐ Enfermedades reumáticas (artritis)
- ☐ Otras (especifique)

ANTECEDENTES PERSONALES

- ☐ No se refiere antecedentes médicos significativos
- ☐ Sufre alguna enfermedad ¿Cual?.....
- ☐ Intervenido quirúrgicamente de. ……..Fecha intervención……..
- ☐ Alérgico a……...
- ☐ Asma alérgica controlada.
- ☐ Intolerancias a……...
- ☐ Fumador de …….cigarrillos /día.
- ☐ Medicación habitual o suplementos vitamínicos……
- ☐ Portados de gafas o lentillas. Miopía / Hipermetropía / Astigmatismo / Estrabismo
- ☐ Portador de plantillas

- Inicia práctica deportiva a los …….años.
- Actualmente entrena…….días a la semana, con una duración de …..min/sesión. Compite……veces/mes.
- *Otras consideraciones:*

10.7 Appendix VII- Anthropometric Measurements



LABSPORTSALUD
FISIOLOGIA DEL EJERCICIO Y VALORACIÓN DE RENDIMIENTO

INFORME DE VALORACIÓN FISIOLÓGICA DE RENDIMIENTO

DATOS PERSONALES

Sujeto		Fecha de la Prueba	1/0/00	F. Nacim.	1/0/00		
Sexo	H/M	Peso	0.0	Talla	0.0	IMC	#DIV/0!

COMENTARIOS

Experiencia en carrera, volumen de entrenamiento semanal, lesiones, historial médico

Email		Teléfono	0

DATOS ANTROPOMÉTRICOS REGISTRADOS

		SOMATOTIPO	DRINKWATER %	kg
IP	#DIV/0!	ENDO I	#DIV/0!	Grasa	#DIV/0!	#DIV/0!
		MESO II	4.50	Muscular	#DIV/0!	#DIV/0!
		ECTO III	#DIV/0!	Óseo	#DIV/0!	#DIV/0!
				Residual	#DIV/0!	#DIV/0!

Pliegues		Perímetros		Diámetros	
Pl. tricipital		P. brazo relajado		D. biacromial	
Pl. subescapular		P. brazo contraido		D. transv. tórax	
Pl. abdominal		P. antebrazo rel.		D. anterop. tórax	
Pl. supraespinal		P. muñeca		D. bileocrestal	
Pl. muslo anterior		P. tórax		D. húmero	
Pl. medial pierna		P. muslo		D. fémur	
		P. pierna			

% Graso Faulkner	5.78			
% Graso YC (H)	¿Sexo?	Sumatorio de 6 pliegues	0	mm
% Graso Carter	2.58	Sumatorio de 4 pliegues	0	mm

COMENTARIOS

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10.8 Appendix VIII- Test Measurements

LABSPORTSALUD
FISIOLOGIA DEL EJERCICIO Y VALORACIÓN DE RENDIMIENTO

INFORME DE VALORACIÓN FISIOLÓGICA DE RENDIMIENTO
RESUMEN DE DATOS Y VALORES MÁXIMOS DE PRUEBA

Peso Final	0.0	Peso Perdido (%_h)	######	Tasa de sudoración (litros_h)	######
Peso Perdido %	#DIV/0!	Peso Perdido (kg)	######	Speed Max. (km/h)	17
VO2Max.(ml/kg/min)	0.0	Lactato Max.(mmol/l)	0.0	FC Max.(ppm)	0

Tiempo	0	3	6	9	12	15	18	21	24	29	32
Speed-km/h	Basal	9	10	11	12	13	14	15	16	17	0
Min/km											
Blood Lactate											
RPE											
Tskin °C											
RER											
HR											
VO2 ml/kg/min											
VE											
FATox_g.min											
CHOox_g.min											
FATox_%											
CHOox_%											
FATox_g*h	0	0	0	0	0	0	0	0	0	0	0
CHOox_g*h	0	0	0	0	0	0	0	0	0	0	0

TENSIÓN ARTERIAL

Mediciones	Sistolica	Diastolica	Media	FC
Tumbado	0	0	0	0
Sentado	0	0	0	0
De pie	0	0	0	0
Final	0	0	0	0
Rec'5	0	0	0	0

Valores U.Aeróbico_VT1	
VO2(ml/kg/min)	0.00
FC(ppm)	0
Ritmo (min/km)	
%VO2 Max.	#DIV/0!
%FC Max.	#DIV/0!

Valores U.Anaeróbico_VT2	
VO2(ml/kg/min)	0.00
FC(ppm)	0
Ritmo (min/km)	
%VO2 Max.	#DIV/0!
%FC Max.	#DIV/0!

Valores Max.	
VO2(ml/kg/min)	0.00
FC(ppm)	0
Ritmo (min/km)	17.00
%VO2 Max.	#DIV/0!
%FC Max.	#DIV/0!

SPEED (km/h- min/km)			
Km/h	Min/km	Km/h	Min/km
8	7.30	15	4.00
9	6.40	15.5	3.52
10	6.00	16	3.45
11	5.27	16.5	3.38
11.5	5.12	17	3.31
12	5	17.5	3.25
12.5	4.48	18	3.20
13	4.36	18.5	3.14
13.5	4.27	19	3.09
14	4.17	19.5	3.04
14.5	4.08	20	3

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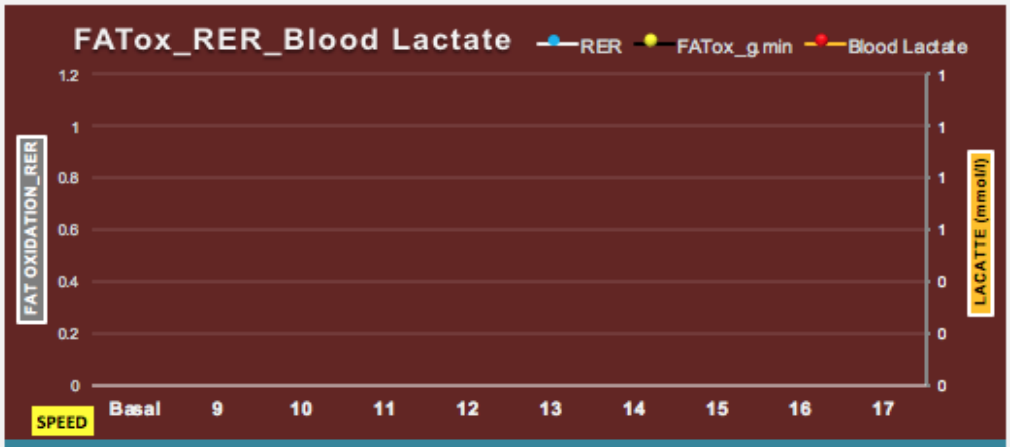
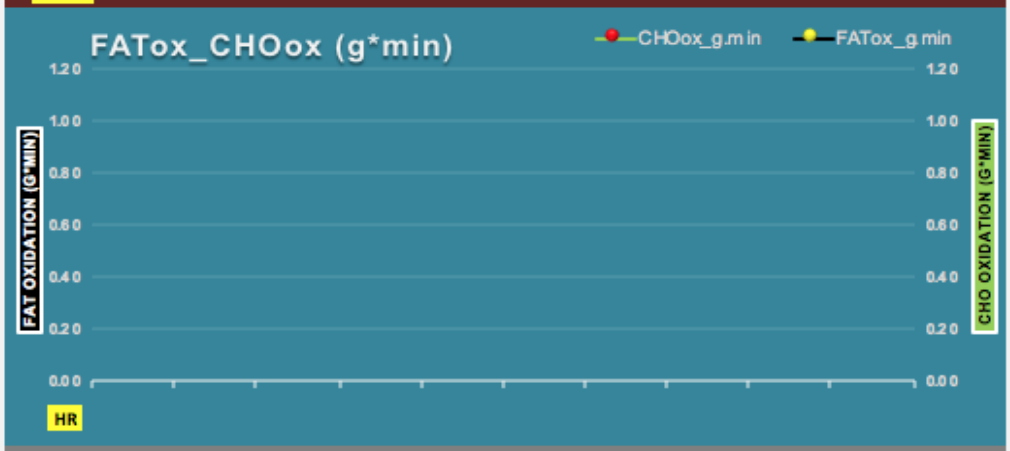
LABSPORTSALUD

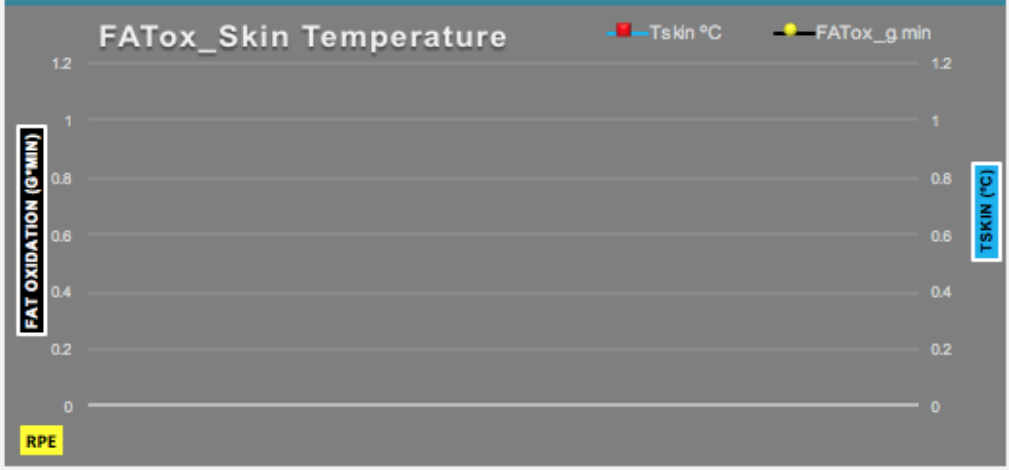
FISIOLOGIA DEL EJERCICIO Y VALORACIÓN DE RENDIMIENTO

INFORME DE VALORACIÓN FISIOLÓGICA DE RENDIMIENTO

GRÁFICAS - INDICADORES DE RENDIMIENTO







*Ratio de Oxidación FAT (Frayn, 1983) - Produccion de Lactato - RER (respiratory exchange ratio)

*Ratio de Oxidación FAT and CHO (Frayn, 1983) - Heart Rate

*Ratio de Oxidación FAT (Frayn, 1983) - Temperatura de la Piel (°C)

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10.9 Appendix IX- Scientific Production

- **Article 2019**

International Journal of *Environmental Research and Public Health*

MDPI

Article

The Dynamic and Correlation of Skin Temperature and Cardiorespiratory Fitness in Male Endurance Runners

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check for updates

Abstract: During endurance exercise, skin temperature (Tsk) plays a fundamental role in thermoregulatory processes. Environmental temperature is the biggest determinant of the Tsk. During exercise, the response of the skin temperature might be influenced by aerobic fitness (VO_{2peak}). The aim of this study was to analyze and compare the dynamic of Tsk in high (HF) and moderately (MF) fit endurance runners during a progressive maximal stress test. Seventy-nine male endurance runners were classified into HF (n = 35; VO_{2peak} = 56.62 ± 4.31 mL/kg/min) and MF (n = 44; VO_{2peak} = 47.86 ± 5.29 mL/kg/min) groups. Tsk and cardiovascular data were continuously monitored during an incremental exercise, followed by a recovery period of five minutes. Results revealed that the MF group exhibited lower VO_{2peak}, $Speed_{peak}$, ventilation (VE), muscle mass %, and higher BMI and fat mass % than the HF group (all $p < 0.001$). HF had significantly higher Tsk at baseline, and at 60% and 70% of peak workload (all $p < 0.05$). Tsk_{peak} correlated with age, fat mass %, muscle mass %, VO_{2peak}, $Speed_{peak}$, HR and VE (all $p < 0.05$). These findings indicate that VO_{2peak} was positively associated with increased Tsk during incremental exercise in male endurance runners.

Keywords: skin temperature; aerobic fitness; endurance

1. Introduction

The ability to thermoregulate is essential for adapting to exercise demands, minimizing changes in body core temperature (Tc) and maintaining physiological homeostasis [1]. Changes in temperature can be perceived at different areas of the human body, where the most critical changes occur for the skin, muscle, and core tissues (i.e., of the rectal, visceral, and esophageal) [2]. Skin temperature (Tsk) plays a fundamental role in body temperature regulation, providing negative and positive auxiliary feedback to the thermoregulatory system. Tsk is the result of the balance between metabolic heat production, heat dissipation to the environment, and tissue temperature [3]. This balance is influenced mainly by the responses of Tc, environment temperature and complex relationships between cutaneous vasodilation and sweating, which facilitate the heat exchange with the environment [4,5]. The delivery of heat from the deeper parts of the body to the skin is accomplished primarily by blood circulation [6], which implies that Tsk can be used as an index to predict thermal changes during exercise [7,8].

During exercise, the impact of environmental conditions influences the skin thermoregulatory response and capacity for heat exchange with the environment [9].

Thermogenesis, from exercise is associated with large hemodynamic changes involving multiple thermoregulatory processes. These changes are reflected in the skin temperature response during exercise [6], and represent a good indication of whether physiological mechanisms are functioning properly, which are vital to the maintenance of thermal homeostasis.

Thermoregulation is the major process that governs skin blood flow (SkBF) in humans [10] and several studies use SkBF to describe and characterize the skin thermoregulatory response to exercise [6,11]. Several studies had been proposed to estimate the skin thermoregulatory response from SkBF and analyze relationship between Tsk and SkBF during exercise [12]. Kenney and Johnson [13] found that the modification of cutaneous blood flow during exercise depends on the individual level of vasodilation and vasoconstriction. The vasoconstrictor response decreases Tsk, induced by a reduction in SkBF; conversely, the vasodilator response leads to increase Tsk, associated with a substantial increase in SkBF. Accordingly, there is a close link between thermoregulatory vasodilation and increased SkBF (together with sweating), which is essential for heat dissipation during exercise [14].

The relationship of skin thermoregulatory response with Tc, SkBF and environment are modulated by several factors, such as sex [15], an individual's acclimatization state [16], environmental conditions [17], body composition [18], aging [15], circadian rhythms [19], the wearing of protective clothing [2], hydration status and/or diet [20,21], lifestyle [22], physiological characteristics [23,24], and relevant to this study, physical conditioning [10,25,26].

Obviously, the thermogenesis associated with aerobic exercise is a challenge to thermoregulation [10]. In fact, recent studies show that aerobic fitness modifies and improves the thermoregulatory control of SkBF, as manifested by a greater augmentation of skin perfusion for the same increase in core temperature and workload in athletes, in comparison with sedentary subjects [10,11,27,28].

Aerobic fitness level is an important determinant in the health status of individuals of any age. It was reported that the maximum oxygen consumption (VO_{2peak}) decreases by about 7% per decade [29]. Based on this discovery, the decrease in skin thermoregulatory capacity, associated with reduced SkBF, may be related to the decline in VO_{2peak} [30]. However, other studies reported that fitness level, associated with regular endurance-type exercise, can induce partial acclimation [16,31] and thereby improve the ability to thermoregulate, enhancing the skin vasodilation response during exercise [11,32]. Boegli [10] concluded that endurance training modifies the skin thermoregulatory response, as manifested by a greater augmentation of skin perfusion and maintenance of active cutaneous vasodilation during exercise [11,28]. Other studies [11,33,34] also reported that physical endurance training and increased VO_{2peak} improve skin thermoregulatory response, which appears to be one of the main elements needed for an effective thermoregulatory active vasodilation response, as well as increased SkBF [30,35]. Moreover, Richmond, Davey, Griggs, and Havenith (2014) [36], found that there was an association between enhanced SkBF response and increased VO_{2peak} after exercise training in older subjects. These changes in VO_{2peak} seemed to affect Tsk responses. Périard et al. (2001) [16] also postulated that a better skin thermoregulatory response during endurance exercise could indicate a higher cardiorespiratory fitness level, despite aging.

Despite these discoveries, Tsk response remains unstudied as an independent parameter in the control of body temperature during exercise [37]. Little attention has been paid to Tsk response, resulting in a key factor in providing better insight into the behavior of the thermoregulatory system during exercise. In this sense, it is unclear if the level of aerobic fitness can influence Tsk response, irrespective of age [38,39]. To the best of our knowledge, the dynamic of Tsk during a maximal stress test and its association with cardiorespiratory fitness in male endurance runners has not been directly studied. Therefore, the main objective of the present study was to analyze and compare the dynamic of Tsk during a maximal stress test in both high (HF) and moderately (MF) fit male endurance runners. Secondly, we analyzed the correlations between Tsk and cardiorespiratory variables. It was hypothesized that HF runners have the ability to maintain higher Tsk responses than MF runners during maximal exercise.

2. Materials and Methods

2.1. Study Design and Participants

The present study followed a cross-sectional research design. A total of 79 trained male endurance runners participated in this study from running and triathlon teams. Participants were eligible for the study if they met the following inclusion criteria: all subjects had to be at an aerobic fitness level ≥40th percentile, based on the American College of Sports Medicine (ACSM) age-specific cardiorespiratory fitness classification [40]; regular running training, at least three times/week and a minimum of 120–150 min/week; having competed in an endurance event (>5 km) within 3 months prior to the study; and ≥3 years of competitive running experience [41]. All subjects were nonsmokers, deemed healthy (assessed by completion of a general health questionnaire), with no known cardiovascular or metabolic disorders, and were not taking medication that had the potential to impact cardiovascular or thermoregulatory function. All significant inclusion criteria were the same for all runners, except age, which was 18–50 years.

Participants were divided into two groups: the HF group was >80th percentile and the MF group was ≤80th percentile based on the ACSM age-specific cardiorespiratory fitness classification. The HF (n = 35; age 36 ± 8 years) and MF (n = 44; age 37 ± 9 years) participants performed a maximal exercise test on a treadmill. Before beginning the study, the participants had the protocol explained to then, as well as the testing procedures and time required for the study. All the participants signed an informed consent form. The procedures of this study followed the Helsinki guidelines [42] for ethical behavior and was approved by the local Human Research Ethics Committee of Blanquerna, University of Ramon Llull.

2.2. Experimental Procedure

Because external and internal factors have an influence on the day of the test, in order to measure Tsk under similar conditions, the following characteristics were used as exclusion criteria and participants were asked not to: (a) smoke or drink alcohol at least 12 h before the test; (b) sunbathe or be exposed to UV rays; (c) use body lotions and creams; (d) carry out high-intensity or exhaustive exercise < 24 h before the test; (e) eat at least 2 h before the test and refrain from having a heavy meal; (f) drink coffee or stimulants 2 h before the test; and (g) use medications, such as antipyretics or diuretics, or any dietary supplement that could potentially interfere with water homeostasis and body temperature in the previous two weeks. Every participant was measured at a similar time in order to reduce the intra-subject effect of the circadian cycle. Finally, heat acclimation can influence the overall control of Tsk during exercise [43]. Consequently, we decided to perform our study in winter and spring, avoiding the possible effects of heat acclimation during the warmer season.

2.3. Anthropometric Measurements

Body mass was measured to the nearest 0.1 kg on a digital scale (Seca 861, Hamburg, Germany), with the subject wearing lightweight clothing and no shoes. Body height was measured using a stadiometer to the nearest 0.1 cm (Seca 225, Seca, Hamburg, Germany). Body mass index (BMI) (kg/m^2) was calculated using body mass and body height, following the recommendations of the International Society for the Advancement of Kinanthropometry [44].

Body density was estimated using the seven site skinfold equation (chest, axilla, subscapular, midaxillary, triceps, abdominal and thigh) developed by Jackson and Pollock [45]. Skinfold measurements were taken on the right side of the body three times by the same researcher using a Holtain skinfold caliper (Holtain Ltd., Walles, UK) and following the ISAK guidelines [44]. Body fat percentage (%) was calculated using the Siri equation [46], with muscle mass percentage determined thereafter. Muscle mass percentage (%) was determined together with bone and organs percentages using the equation of the sum of seven perimeters (arm, contracted arm, forearm, wrist, chest, upper thigh, medial thigh and calf) and 6 diameters (biacromial iliac spine, breadth, chest,

humerus, femur, anterior-posterior thoracic and transverse thoracic) [47]. Finally, all tests were performed in the morning between 9 am to 12 pm to reduce the intra-subject effect of the circadian cycle. The test was carried out in a controlled environment, where conditions were maintained at 22 ± 1 °C and 50 ± 5% relative humidity.

2.4. Cardiorespiratory Fitness Assessment

Each participant performed an incremental test on a treadmill (Quasar model, HP Cosmos sports and medical gmbh, Nussdorf-Traunstein, Germany). During the test, Tsk (using Biopac Student Lab software) was monitored, as were cardio-vascular and ventilation responses using a gas analyzer. Oxygen consumption (VO_2), CO_2 production (VCO_2), ventilation (VE) and the respiratory exchange ratio (RER = VCO_2/VO_2) were measured breath-by-breath with an automatic gas analysis system (Ergospirometer, Powercube-Ergo, Gansborn Medizine Electronic GmbH, Niederlaur, Germany). During the test, participants started at a speed of 7 km/h, which increased by 1 km/h every 2 min until exhaustion. The participants performed the test at a constant slope (1.5%). All cardiorespiratory variables, rate of perceived exertion (RPE) and Tsk were monitored at rest, during exercise and during the 5 min recovery period. The twelve lead electrocardiograms (CardioScan v.4.0, DM Software, Staline, NV, USA) and heart rate (HR) (Polar RS800CX, Polar Electro, Lake Success, NY, USA) were monitored continuously during the test and five minutes of the recovery period. Perception of fatigue was reported by means of a 6–20 point Borg scale [48].

2.5. Skin Temperature Assessment

On the day of test, subjects reported normal hydration. Skin temperature was continuously recorded by a Tsk sensor (thermistor sensor, TSD202D, Biopac Systems Inc., Goleta, CA, USA) that was placed on the left pectoralis muscle 2.5 cm medial and 2.5 cm above the nipple. Accuracy and precision of the device model is ± 0.2 °C. Before monitoring Tsk, participants were acclimated to the environment by standing in the room for 15 min. During the incremental test, Tsk data was recorded every half second and the mean value of each ten seconds was used for data analysis (Biopac Student Lab Analysis software, Biopac Systems Inc., Goleta, CA, USA).

2.6. Statistical Analysis

After checking the normal distribution of the variables (Kolmogorov-Smirnov test), a one-way ANOVA with post-hoc Bonferroni test was used to determine between-group differences. Pearson's r correlation was used to analyze the associations between Tsk and cardiorespiratory variables.

Finally, a multiple linear regression identifying significant variables was developed with Tsk used as the response variable. The explanatory variables were those found to be significantly correlated ($p < 0.05$) with Tsk, using a linear relationship. Multicollinearity was checked with the variance inflation factor (VIF), which needs to be below 10 for all predictor variables [49].

The critical values for statistical significance were assumed to be at an alpha level of less than 0.05. Statistical analyses were conducted using the Statistical Package for the Social Sciences (IBM SPSS, v 22.0, Chicago, IL, USA).

3. Results

3.1. Participant Characteristics and Cardiorespiratory Assessments

Table 1 shows general and physiological characteristics of the participants. All subjects were similar in height and age. Body weight and BMI were significantly higher in the MF group ($p = 0.001$), compared with the subjects of the HF group. The HF group had a higher muscle mass % and a lower fat mass % than the MF group (all $p < 0.001$).

Cardiovascular Response

VO_{2peak} was significantly higher in the HF group than the MF group (56.62 ± 4.31 vs. 47.86 ± 5.29 mL/kg/min, $p < 0.05$). Also, both the maximal speed reached during the test and VE were higher in the HF group compared with the MF group (all $p < 0.001$). Significant differences in peak HR (HR_{peak}) and peak RER (RER_{peak}) were not found.

Table 1. General characteristics of whole sample and of the two study groups.

Variables	$n = 79$	HF ($n = 35$)	MF ($n = 44$)	p-Value
Age (years)	36 ± 9	36 ± 8	37 ± 9	0.734
Height (cm)	177 ± 0.1	176 ± 0.5	178 ± 0.6	0.189
Weight (kg)	75.62 ± 7.56	72.72 ± 5.67	77.93 ± 8.12	0.001
BMI (kg/m^2)	24.03 ± 2.11	23.23 ± 1.50	24.68 ± 2.32	0.001
Fat mass (%)	14.04 ± 4.52	11.69 ± 3.2	15.91 ± 4.59	<0.001
Muscle mass (%)	45 ± 3.48	46.51 ± 2.70	43.80 ± 3.61	<0.001
VO_{2peak} (L/min)	3.82 ± 0.38	4.02 ± 0.35	3.65 ± 0.33	0.001
VO_{2peak} (mL/kg/min)	51.74 ± 6.54	56.62 ± 4.31	47.86 ± 5.29	<0.001
HR (beat/min)	181 ± 9	182 ± 8	181 ± 11	0.554
RER (VCO_2/VO_2)	1.05 ± 0.05	1.05 ± 0.05	1.04 ± 0.05	0.590
VE (L/m)	136 ± 20	145 ± 21	129 ± 17	<0.001
$Speed_{peak}$ (km/h)	15.91 ± 1.78	16.98 ± 1.50	15.06 ± 1.50	<0.001
Time spent (sec)	1200 ± 212	1329 ± 181	1099 ± 180	<0.001

Values are means ± SD. Abbreviations: BMI (body mass index), VO_{2peak} (peak oxygen consumption), HR (heart rate), RER (respiratory exchange ratio), VE (ventilation), $Speed_{peak}$ (peak speed), HF (highly fit endurance runners), MF (moderately fit endurance runners).

3.2. Skin Temperature Measurements

Results from the analysis of Tsk are shown in Table 2. These results provide the mean Tsk responses at rest (baseline), during exercise, and after exercise during the recovery period. Baseline Tsk was significantly higher ($p = 0.049$) in the HF group compared with the MF group.

Table 2. Skin temperature response in an incremental exercise test until volitional exhaustion in high and moderately fit endurance runners.

Variables	$n = 79$	HF ($n = 35$)	MF ($n = 44$)	p-Value
$Tsk_{baseline}$ (°C)	34.06 ± 0.75	34.24 ± 0.84	33.91 ± 0.74	0.049
Tsk_{peak} (°C)	36.03 ± 0.73	36.20 ± 0.60	35.90 ± 0.79	0.062
Tsk_{final} (°C)	35.59 ± 0.95	35.70 ± 0.77	35.50 ± 1.07	0.322
Tsk variation from $Tsk_{baseline}$ to Tsk_{peak} (°C)	1.97 ± 0.81	1.96 ± 0.65	1.99 ± 0.92	0.833
Tsk variation from $Tsk_{baseline}$ to Tsk_{final} (°C)	1.53 ± 1.03	1.49 ± 0.84	1.56 ± 1.16	0.747
Tsk variation from Tsk_{peak} to Tsk_{final} (°C)	−0.45 ± 0.49	−0.50 ± 0.50	−0.41 ± 0.49	0.413
Time-duration from $Tsk_{baseline}$ to Tsk_{peak} (s)	996 ± 240	1105 ± 244	910 ± 201	<0.001
Time-duration from Tsk_{peak} to Tsk_{final} (s)	204 ± 163	223 ± 159	189 ± 167	0.348
Tsk_{peak} during recovery (°C)	36.25 ± 0.81	36.38 ± 0.79	36.14 ± 0.81	0.187
Tsk_{final} during recovery (°C)	36.12 ± 0.88	36.28 ± 0.87	36 ± 0.88	0.167
Tsk variation from Tsk_{final} at end of exercise to Tsk_{peak} during recovery (°C)	0.71 ± 0.59	0.76 ± 0.56	0.68 ± 0.61	0.557
Tsk variation from Tsk_{final} at end of exercise to Tsk_{final} at end of recovery (°C)	0.60 ± 0.68	0.65 ± 0.56	0.54 ± 0.75	0.452

Values are means ± SD. Abbreviations: Tsk (skin temperature), Tskbaseline (baseline skin temperature), Tskpeak (maximal skin temperature), Tskfinal (skin temperature at the end of exercise/recovery), HF (highly fit endurance runners), MF (moderately fit endurance runners).

Values of Tsk at each percentage of peak workload during the incremental test are shown in Figure 1. Mean Tsk and standard error (SEM) of both groups during exercise are shown in Figure 1. This figure also shows mean Tsk and SEM for each group during the recovery period. During the test,

Tsk values were higher in the HF group compared with the MF group (Figure 1). However, these were not statistically significant differences. As the duration and intensity of the test increased, the maximal Tsk (Tsk_{peak}) reached was lower in the MF group compared with the HF group throughout exercise (35.90 ± 0.79 vs. 36.20 ± 0.60 °C, respectively). Nevertheless, there were not statistically significant differences between groups (Figure 1). Similarly, during the recovery period, the peak values of Tsk were higher in the HF group compared with the MF group (Figure 1). Both groups reached their Tsk_{peak} during recovery. However, there were not statistically significant differences between groups.

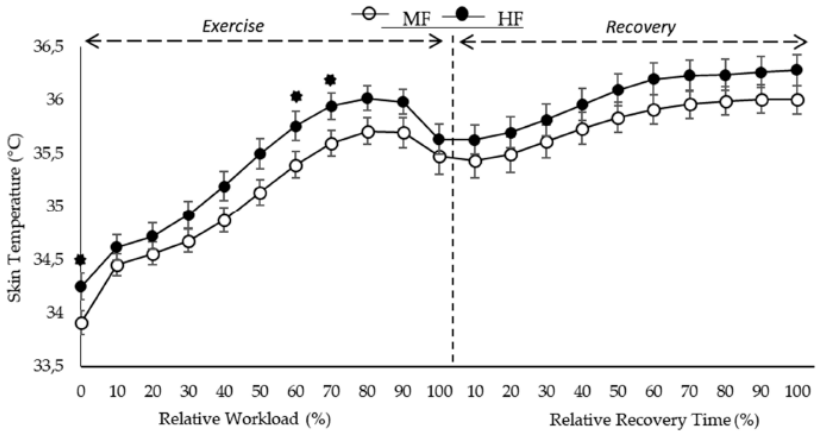

Figure 1. Values are means ± SEM. The black circles represent the highly fit endurance runners (HF) group and the white circles represent the moderately fit endurance runners (MF) group. Significant level was set at $p < 0.05$. Analysis was performed at the end of each relative workload of exercise and recovery period. * Significant differences between highly and moderately fit endurance runners.

During the test, irrespective of Tsk_{peak} and fitness level, the increase of Tsk from $Tsk_{baseline}$ to Tsk_{peak} was not statistically different (1.96 ± 0.65 vs. 1.99 ± 0.92 °C in HF and MF, respectively) during the test. The difference between the Tsk at the beginning of the recovery period and the higher Tsk achieved in this period for both groups was not statistically different (0.65 ± 0.56 vs. 0.54 ± 0.75 °C in HF and MF, respectively) (Figure 1).

Figure 1 also illustrates the mean responses of Tsk for each group during the recovery period, in which there were not significant differences between groups. The comparison of percentage workload (%) of Tsk responses in the HF and MF subjects during the test and recovery period are shown in Figure 1. Throughout the exercise period, the Tsk response for the HF group was significantly higher at baseline ($p = 0.049$), at 60% ($p = 0.048$) and 70% ($p = 0.048$) of peak workload (%) compared with the MF group (Figure 1). There were no other differences in Tsk among the groups during test and recovery periods (Figure 1). The mean values of Tsk at the end of the test were slightly higher in the HF group, nevertheless, the difference among groups was not significant (HF 35.70 ± 0.77 vs. MF 35.50 ± 1.07 °C). During the incremental test both groups reached stable Tsk values (plateau) at 80 to 90% of peak workload (Figure 1). After reaching the plateau, Tsk started decreasing in both groups (HF = 0.50 ± 0.50 vs. LF = 0.41 ± 0.49 °C). The Tsk_{peak} during the recovery period was greater than the Tsk_{peak} reached during exercise in both groups (HF 36.38 ± 0.79 °C vs. MF 36.14 ± 0.81 °C, respectively), and was also greater than the final Tsk value after recovery.

As showed in Table 3, Tsk_{peak} of both group was inversely correlated with fat mass %. On the other hand, Tsk was positively correlated with age, muscle mass %, VO_{2peak}, HR_{peak}, VE_{peak} ($p < 0.05$) and $Speed_{peak}$ ($p = 0.002$) (Table 3).

Table 3. Correlation Coefficients and *p*-values among Tsk_{peak} and Age, BMI, Fat mass %, Muscle mass %, VO_{2peak}, HR_{peak}, RER_{peak}, VE_{peak} and $Speed_{peak}$.

Variables	Correlation Coefficient	*p*-Value
Age, years	0.306	0.006
BMI (kg/m^2)	−147	0.196
Fat mass (%)	−276	0.014
Muscle mass (%)	0.263	0.019
VO_{2peak} (ml/kg/min)	0.299	0.007
HR_{peak} (beat/min)	0.286	0.011
RER_{peak}	−035	0.760
VE_{peak} (L/min)	0.256	0.023
$Speed_{peak}$ (km/h)	0.337	0.002

Values are means ± SD. Abbreviations: Tsk_{peak} (peak skin temperature), BMI (body mass index), VO_{2peak} (peak oxygen consumption), HR_{peak} (peak heart rate), RER_{peak} (peak respiratory exchange ratio), VE_{peak} (peak ventilation) and $Speed_{peak}$ (peak speed).

The multivariate linear regression that was used to identify factors that significantly affected Tsk_{peak} showed that $Speed_{peak}$ had a significant effect on Tsk_{peak}. Although HR_{peak} has positive effects on Tsk_{peak}, no significant difference was found between groups for this variable (Table 4).

Table 4. Variables that affect Peak Skin Temperature (Tsk_{peak}).

Variables	β	β Error	*T*	*p*	VIF
(Constant)	31.668	1.453	21.790	<0.001	-
$Speed_{peak}$	0.111	0.046	2.392	0.019	1.139
HR_{peak}	0.014	0.008	1.694	0.094	1.138

Regression model statistically significant $p = 0.002$; $R^2 = 0.146$. Abbreviations: Tsk_{peak} (peak skin temperature), VIF (variance inflation factor); $Speed_{peak}$ (peak speed) and HR_{peak} (peak heart rate).

4. Discussion

In this study we hypothesized that higher aerobic capacity could be associated with an enhanced response of Tsk in male endurance runners. This hypothesis was tested by comparing two groups of male endurance runners with different levels of aerobic capacity during a maximal exercise test. We observed that the HF group achieve higher Tsk values compared with the MF group, however, the Tsk_{peak} achieved by the groups were not statistically different. As showed in Figure 1, the Tsk dynamic in both groups followed a similar pattern. This may be due to the fact that all subjects were in good physical condition, based on their VO_{2peak} [50]. It is unclear if the observed larger increases in Tsk for the HF group could be due to the fact that subjects with higher aerobic fitness levels may have a better skin thermoregulatory response during exercise.

The Tsk dynamic during the incremental test can be divided into three parts: (i) initial rise to Tsk_{peak}, at around 80% of workload, with an increase in Tsk in the HF and MF groups of 1.96 °C and 1.99 °C, respectively; (ii) a plateau of Tsk at 80% to 90% of peak workload in both groups and, (iii) a decrease in Tsk until the end of exercise. Accordingly, this pattern of increases in Tsk during exercise is consistent with previous observations in individuals with high fitness levels [10]. Our results show that as exercise intensity and VO_2 increase during the test, Tsk increases until 80–90% of peak workload is reached. It is likely that this continuous increase in Tsk is associated with active cutaneous vasodilation, resulting from increased absolute SkBF as exercise intensity increases [25]. At 80% to 90% of peak workload (Figure 1) we observed a plateau of the Tsk on both groups [5]. From this

point, both groups showed a critical and sudden decrease in Tsk, which may likely be associated with decreased SkBF, cutaneous vasodilation and the formation of sweating that will dissipate body heat. Based on previous studies [8], a Tsk plateau at high exercise intensities shows a withdrawal of active vasodilation, which reduces the ability of the athlete to dissipate heat, causing cutaneous vasoconstriction, which is associated with increased adrenergic activity [51]. Our results also seem to confirm the results found by Zontak et al. (1998) [6], in which the rate of decrement of the Tsk was dependent on the intensity of the workload.

During the initial period of the recovery phase, Tsk values increased quickly in both groups due to the need to dissipate heat after exercise. In this phase, our objective was to analyze and compare the behavior and increases in Tsk in both groups. Another study also found similar patterns, and demonstrated that these increases in Tsk reflect the convective transfer of heat from the core to the periphery [37].

Skin temperature is influenced by fitness level, as well as by other variables such as body composition, fat and muscle mass %, age, and other cardiovascular variables, like VE and HR. Aerobic fitness level, along with age, appear to be the most important limiting factors in the cutaneous vasodilation response to exercise [23]. Therefore, physical inactivity and aging contribute to reducing VO_{2peak}, which decreases heat dissipation capacity and, consequently, body temperature control during strenuous exercise [52,53]. The MF subjects had a higher BMI, fat mass %, and lower muscle mass and VO_{2peak} (Table 1). These variables and age itself have all been found independently and negatively associated with Tsk_{peak}, which is related to maximal cutaneous vasodilation [10].

The result of the multiple linear regression shows that $Speed_{peak}$ has a significant effect on the Tsk_{peak} of the participants. Nevertheless, these results should be interpreted with caution as only 15% of the variance of the Tsk_{peak} is explained by the model.

The results of our study are similar to the results of Merla et al. (2005) [4], in which changes in Tsk during exercise were associated with highly trained individuals, due to a major activation of the sympathetic active vasodilator system and better thermal adaptations [54]. These findings may be linked to direct and substantial vasodilation of blood vessels [55], which contributes importantly to SkBF, helping to reach high Tsk values. Stapleton et al. [30] also found that higher levels of aerobic fitness were associated with an increased rate of Tsk for a given increase in mean body temperature and exercise intensity.

We conclude that despite between-group differences in fitness level, our results did not show significant differences in Tsk increases between both groups. However, we provide evidence that a moderate to high fitness level may enhance skin thermoregulation response, at least in the torso where the skin temperature sensor was located. This influence of aerobic capacity shows a close relationship between Tsk and cardiovascular responses to relative exercise intensity.

Based on previous studies [56,57] and in our findings, it is clear that the response of the Tsk plays an important role in the thermoregulatory process during maximal exercise. Despite that Tsk is an important measure and contributor for the thermoregulation and aerobic performance, it should be taken into account that Tsk is mainly a consequence of other important factors such as Tc, SkBF and environmental temperature [12].

It is important to acknowledge the limitations of this study. Firstly, between-group difference on Tsk_{peak}, which was ~0.3 °C, should be interpreted with caution as the accuracy of the device assessing the Tsk is ±0.2 °C. Nevertheless, all participants were assessed by using the same device and methodology, so all assessments may present the same measurement error. Secondly, this is a cross-sectional study and did not study the effects of specific endurance training programs, where the differences between both groups may be due to different physiological capacity to control the skin thermoregulatory response during exercise. Thirdly, Tc was not measured during the incremental test. Core temperature could show that differences in body temperature are due to differences in fitness level, as reported by the literature [5,30,51]. The relationship of this thermal behavior with the Tc and other metabolic responses could help to better determine thermoregulatory response during exercise.

Hence, it would be of interest to study the relationship of skin thermal behavior related to Tc and other metabolic responses during graded and progressive exercise in different populations. Finally, sweat on the skin surface at the end of exercise could have influenced Tsk data and should also be considered a limitation of the present study. Ammer (2009) [58] suggested that a film of water on the skin may work as a filter and, therefore, could lead to an underestimation of thermal data.

The runners who participated in this study presented a different range of aerobic fitness and practiced different endurance sports activities, such as distance running, mountain running and triathlon. This is a strength when it comes to ensuring that the results of the present study not only apply to runners with high aerobic fitness, but also to those exercising at a high recreational level. Future studies should study the skin thermoregulatory response in female endurance runners, and compare the results with those of their male counterparts. Also, it should be interesting to compare and analyze the difference between peripheral and core temperature of male and female runners.

5. Conclusions

The main findings of the present study are that VO_{2peak} is positively associated with Tsk_{peak} rates. However, our results do not show that a higher level of aerobic fitness in male endurance runners contributes to achieve higher Tsk, or a greater thermoregulatory or physiological capacity to dissipate heat during exercise. These observations are of considerable interest in view of our results, which also found that $Speed_{peak}$ is a primary contributor to the increased Tsk response. The dynamic response of Tsk to exercise reflects the balance of hemodynamic and thermoregulatory processes, and may serve as a tool for assessing the integrity of these mechanisms as part of the circulatory system, which interacts with the thermal and hemodynamic responses. These findings might able to contribute to enhancing performance in various endurance or even team sports, where the relationship between aerobic endurance and thermal control can limit endurance performance. More research and future studies are required to determine how the thermoregulatory control of Tsk could substantially affect aerobic performance.

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Abstracts

- **2020 (American College of Sports Medicine, ACSM)- San Francisco (USA) (*In review*)**

Relationship between Skin Temperature Response and Metabolic Flexibility in Endurance Runners and Professional Soccer Players

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During endurance exercise, skin temperature (Tsk) plays a fundamental role in thermoregulation and might be influenced by aerobic fitness (VO_{2peak}) and metabolic flexibility. Aim: to compare the Tsk response and metabolic flexibility by measuring blood lactate ([La^-]), fat oxidation (FATox) and carbohydrate oxidation (CHOox) rates. Also, we analyzed the correlation between these variables in both runner groups and the soccer player group. Methods: we used indirect calorimetry and [La^-] measurements, and monitored the Tsk response in highly trained (HT) (n= 22) endurance runners, moderately active (MA) (n= 20) runners, and professional soccer (PS) (n= 23) players during an incremental maximal treadmill test with stages of 3 mins, followed by a recovery period of 5 mins. Results: there were correlations between Tsk with FATox and CHOox rates, and [La^-] for all data points of all groups (all $p < 0.001$). The average FATox rates and Tsk measurements were significantly higher in the HT (0.30±0.08 $g.min^{-1}$; 35.55±0.48 °C) and PS (0.23±0.13 $g.min^{-1}$; 35.62±0.50 °C) groups compared with the MA (0.19±0.11 $g.min^{-1}$; 34.98±0.50 °C) group, respectively. Conclusions: our data showed that higher $FATox_{average}$ in the HT and PS groups was associated with an increased $Tsk_{average}$ compared with the MA group. We also found that the HT and PS groups had a higher VO_{2peak}, average FATox rates and a lower average of [La^-], suggesting that the quantities of substrate (Fat or CHO) used during the test was different than the quantities used by the MA group. These results should be taken into account for training and nutritional strategies for enhancing endurance performance.

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Keywords: skin temperature; metabolic flexibility; endurance performance.

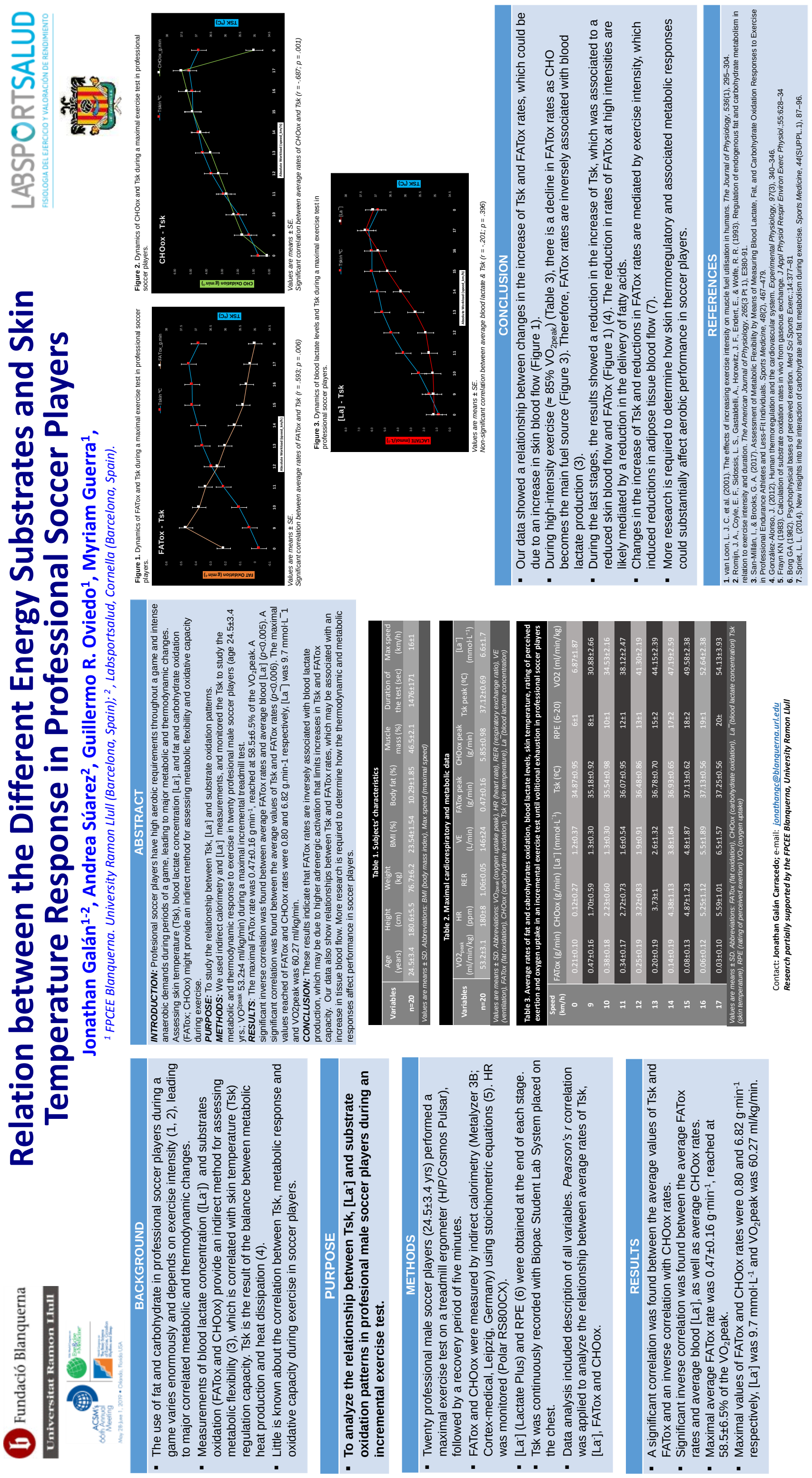
Table 1. Anthropometrics, cardiorespiratory, metabolic and skin temperature data of the three study groups.

Variables	HT (n=22)	MA (n=20)	PS (n=23)	*F*	*p*-value
Characteristics and Anthropomety					
Age (years) *; **; ***	33 ± 5	37 ± 5	25 ± 4	41.484	< .001
Height (cm)	176 ± 6.7	176 ± 7.8	181 ± 5.7	3.828	.027
Weight (kg) **	70.68 ± 6.67	75.65 ± 7.41	75.94 ± 5.80	4.374	.017
BMI (kg/m^2) *; ***	22.86 ± 1.33	24.39 ± 1.48	23.25 ± 1.16	7.457	.001
Fat mass (%) *; ***	9.19 ± 1.38	12.11 ± 2.32	9.66 ± 1.39	17.106	< .001
VO_{2peak} (ml/kg/min) *; **; ***	58.57 ± 2.33	49.07 ± 4.67	53.34 ± 3.67	35.901	< .001
HR (beat/min)	181 ± 9	183 ± 8	180 ± 7	0.662	.520
VE (L/m)	148 ± 16	135 ± 24	142 ± 23	1.897	.159

$Speed_{peak}$ *; **; ***	17.41 ± 0.80	14.45 ± 1.14	16.26 ± 0.81	54.552	**< .001**
Metabolic variables					
$FATox_{baseline}$ (g/min) ***	0.19 ± 0.05	0.14 ± 0.06	0.21 ± 0.09	4.915	**.010**
$FATox_{max}$ (g/min) **	0.63 ± 0.13	0.51 ± 0.23	0.49 ± 1.17	3.935	**.025**
$FATox_{average}$ (g/min) *; ***	0.30 ± 0.08	0.19 ± 0.12	0.23 ± 0.14	4.299	**.018**
$FATox_{final}$ (g/min) during recovery	0.03 ± 0.03	0.01 ± 0.02	0.03 ± 0.05	2.144	.126
$CHOox_{max}$ (g/min)	6.07 ± 0.83	5.76 ± 1.20	5.72 ± 0.98	0.811	.449
$[La^-]_{peak}$ (mmol/L) ***	7.19 ± 1.21	8.25 ± 1.90	7.08 ± 1.22	3.963	**.024**
$[La^-]_{average}$ (mmol/L) *; ***	2.82 ± 0.44	3.97 ± 0.92	3.27 ± 0.80	12.477	**< .001**
$[La^-]_{final}$ (mmol/L) during recovery ***	6.99 ± 1.36	7.77 ± 2.00	6.23 ± 1.59	4.554	**.014**
Skin Temperature					
$Tsk_{baseline}$ (°C) ***	34.13 ± 0.34	33.86 ± 0.54	34.20 ± 0.42	3.428	**.039**
Tsk_{peak} (°C) *; ***	36.68 ± 0.68	35.97 ± 0.66	36.96 ± 0.79	10.786	**< .001**
$Tsk_{average}$ (°C) *; ***	35.55 ± 0.48	34.98 ± 0.50	35.62 ± 0.50	10.276	**< .001**

Note: values are means ± SD. Abbreviations: BMI (body mass index), VO_{2peak} (peak oxygen consumption), HR (heart rate), VE (ventilation), $Speed_{peak}$ (peak speed), $FATox_{max}$ (maximal fat oxidation), $FATox_{final}$ (final fat oxidation), $CHOox_{max}$ (maximal carbohydrate oxidation, $[La^-]_{peak}$ (maximal blood lactate concentrations), $[La^-]_{final}$ (final blood lactate concentrations) $Tsk_{baseline}$ (baseline skin temperature), Tsk_{peak} (maximal skin temperature), HT (high trained endurance runners), MA (moderately active runners) and PS (professional soccer players). * Significant difference (p 0.05) between HT vs. MA. ** Significant difference (p 0.05) between HF vs. PS. *** Significant difference (p 0.05) between MA vs PS.

- **2019 (American College of Sports Medicine, ACSM)- Orlando (USA)**



Relation between the Different Energy Substrates and Skin Temperature Response in Professional Soccer Players

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BACKGROUND

- The use of fat and carbohydrate in professional soccer players during a game varies enormously and depends on exercise intensity (1, 2), leading to major correlated metabolic and thermodynamic changes.
- Measurements of blood lactate concentration ([La⁻]) and substrates oxidation (FATox and CHOox) provide an indirect method for assessing metabolic flexibility (3), which is correlated with skin temperature (Tsk) regulation capacity. Tsk is the result of the balance between metabolic heat production and heat dissipation (4).
- Little is known about the correlation between Tsk, metabolic response and oxidative capacity during exercise in soccer players.

PURPOSE

- **To analyze the relationship between Tsk, [La⁻] and substrate oxidation patterns in profesional male soccer players during an incremental exercise test.**

METHODS

- Twenty professional male soccer players (24.5±3.4 yrs) performed a maximal exercise test on a treadmill ergometer (H/P/Cosmos Pulsar), followed by a recovery period of five minutes.
- FATox and CHOox were measured by indirect calorimetry (Metalyzer 3B; Cortex-medical, Leipzig, Germany) using stoichiometric equations (5). HR was monitored (Polar RS800CX).
- [La⁻] (Lactate Plus) and RPE (6) were obtained at the end of each stage.
- Tsk was continuously recorded with Biopac Student Lab System placed on the chest.
- Data analysis included description of all variables. *Pearson's r* correlation was applied to analyze the relationship between average rates of Tsk, [La⁻], FATox and CHOox.

RESULTS

- A significant correlation was found between the average values of Tsk and FATox and an inverse correlation with CHOox rates.
- Significant inverse correlation was found between the average FATox rates and average blood [La⁻], as well as average CHOox rates.
- Maximal average FATox rate was 0.47±0.16 g·min⁻¹, reached at 58.5±6.5% of the VO_2peak.
- Maximal values of FATox and CHOox rates were 0.80 and 6.82 g·min⁻¹ respectively, [La⁻] was 9.7 mmol·L⁻¹ and VO_2peak was 60.27 ml/kg/min.

ABSTRACT

INTRODUCTION: Profesional soccer players have high aerobic requirements throughout a game and intense anaerobic demands during periods of a game, leading to major metabolic and thermodynamic changes. Assessing skin temperature (Tsk), blood lactate concentration [La⁻], and fat and carbohydrate oxidation (FATox; CHOox) might provide an indirect method for assessing metabolic flexibility and oxidative capacity during exercise.
PURPOSE: To study the relationship between Tsk, [La⁻] and substrate oxidation patterns.
METHODS: We used indirect calorimetry and [La⁻] measurements, and monitored the Tsk to study the metabolic and thermodynamic response to exercise in twenty profesional male soccer players (age 24.5±3.4 yrs.; VO2peak 53.2±4 ml/kg/min) during a maximal incremental treadmill test.
RESULTS*:* The maximal FATox rate was 0.47±0.16 g·min⁻¹, reached at 58.5±6.5% of the VO_2peak. A significant inverse correlation was found between average FATox rates and average blood [La⁻] (p<0.005). A significant correlation was found between the average values of Tsk and FATox rates (p<0.006). The maximal values reached of FATox and CHOox rates were 0.80 and 6.82 g.min-1 respectively, [La⁻] was 9.7 mmol·L⁻1 and VO2peak was 60.27 ml/kg/min.
CONCLUSION: These results indicate that FATox rates are inversely associated with blood lactate production, which may be due to higher adrenergic activation that limits increases in Tsk and FATox capacity. Our data also show relationships between Tsk and FATox rates, which may be associated with an increase in tissue blood flow. More research is required to determine how the thermodynamic and metabolic responses affect performance in soccer players.

Table 1. Subjects' characteristics

Variables	Age (years)	Height (cm)	Weight (kg)	BMI (%)	Body fat (%)	Muscle mass (%)	Duration of the test (sec)	Max speed (km/h)
n=20	24.5±3.4	180.6±5.5	76.7±6.2	23.54±1.54	10.29±1.85	46.5±2.1	1476±171	16±1

Values are means ± SD. Abbreviations: BMI (body mass index), Max speed (maximal speed)

Table 2. Maximal cardiorespiratory and metabolic data

Variables	$VO2_{peak}$ (ml/min/kg)	HR (ppm)	RER	VE (L/min)	FATox peak (g/min)	CHOox peak (g/min)	Tsk peak (ºC)	[La⁻] (mmol·L⁻¹)
n=20	53.2±3.1	180±8	1.06±0.05	146±24	0.47±0.16	5.85±0.98	37.12±0.69	6.6±1.7

Values are means ± SD. Abbreviations: VO_{2peak} (oxygen uptake peak), HR (heart rate), RER (respiratory exchange ratio), VE (ventilation), FATox (fat oxidation), CHOox (carbohydrate oxidation), Tsk (skin temperature), La⁻(blood lactate concentration)

Table 3. Average rates of fat and cabrohydrates oxidation, blood lactate levels, skin temperature, rating of perceived exertion and oxygen uptake in an incremental exercise test until volitional exhaustion in professional soccer players

Speed (km/h)	FATox (g/min)	CHOox (g/min)	[La⁻] (mmol·L⁻¹)	Tsk (ºC)	RPE (6-20)	VO2 (ml/min/kg)
0	0.21±0.10	0.12±0.27	1.2±0.37	34.87±0.95	6±1	6.87±1.87
9	0.47±0.16	1.70±0.59	1.3±0.30	35.18±0.92	8±1	30.88±2.66
10	0.38±0.18	2.23±0.60	1.3±0.30	35.54±0.98	10±1	34.53±2.16
11	0.34±0.17	2.72±0.73	1.6±0.54	36.07±0.95	12±1	38.12±2.47
12	0.25±0.19	3.22±0.83	1.9±0.91	36.48±0.86	13±1	41.30±2.19
13	0.20±0.19	3.73±1	2.6±1.32	36.78±0.70	15±2	44.15±2.39
14	0.14±0.19	4.38±1.13	3.8±1.64	36.93±0.65	17±2	47.19±2.59
15	0.08±0.13	4.87±1.23	4.8±1.87	37.13±0.62	18±2	49.58±2.38
16	0.06±0.12	5.25±1.12	5.5±1.89	37.13±0.56	19±1	52.64±2.38
17	0.03±0.10	5.59±1.01	6.5±1.57	37.25±0.56	20±	54.13±3.93

Values are means ± SD. Abbreviations: FATox (fat oxidation), CHOox (carbohydrate oxidation), La⁻(blood lactate concentration) Tsk (skin temperature), RPE (rating of perceived exertion) VO_2 (oxygen uptake)

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Figure 1. Dynamics of FATox and Tsk during a maximal exercise test in professional soccer players.

Values are means ± SE.
Significant correlation between average rates of FATox and Tsk (r = .593; p = .006)

Figure 2. Dynamics of CHOox and Tsk during a maximal exercise test in professional soccer players.

Values are means ± SE.
Significant correlation between average rates of CHOox and Tsk (r = -.687; p = .001)

Figure 3. Dynamics of blood lactate levels and Tsk during a maximal exercise test in professional soccer players.

Values are means ± SE.
Non-significant correlation between average blood lactate & Tsk (r = -.201; p = .396)

CONCLUSION

- Our data showed a relationship between changes in the increase of Tsk and FATox rates, which could be due to an increase in skin blood flow (Figure 1).
- During high-intensity exercise (≈ 85% VO_{2peak}) (Table 3), there is a decline in FATox rates as CHO becomes the main fuel source (Figure 3). Therefore, FATox rates are inversely associated with blood lactate production (3).
- During the last stages, the results showed a reduction in the increase of Tsk, which was associated to a reduced skin blood flow and FATox (Figure 1) (4). The reduction in rates of FATox at high intensities are likely mediated by a reduction in the delivery of fatty acids.
- Changes in the increase of Tsk and reductions in FATox rates are mediated by exercise intensity, which induced reductions in adipose tissue blood flow (7).
- More research is required to determine how skin thermoregulatory and associated metabolic responses could substantially affect aerobic performance in soccer players.

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- **2018 (American College of Sports Medicine, ACSM) – Minneapolis (USA)**

- **2017 (European College of Sports Science, ECSS) – Metropolis Ruhr (Germany)**

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Corporació Mèdica Catalana

Relation between Thermoregulation and VO2max in male endurance athletes

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Introduction: The ability of thermoregulation is essential for adapting to exercise demands. Good adaptation is needed to maintain an adequate performance in endurance sports (ES) and prevent fatigue. The aim of this study was to analyze the relationship between aerobic power (VO2peak) and the thermoregulatory variability of skin temperature (Ts) in trained male adults of endurance sports during a progressive treadmill test, and study if a better thermoregulatory variability could determine a better functional capacity.

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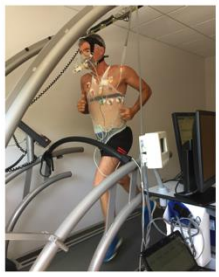
Relation between Thermoregulation and VO2max in male endurance athletes

Methods: Twenty-three trained male adults of ES (age=38,5 (7,8) years; height= 177,9 (6,2cm)) with the same activity level, participated in the study after signing an informed consent. Each participant performed a progressive increasing treadmill test until exhaustion in a room with a stable humidity of 40-60% and a temperature of 24 ºC.



During the test, skin temperature (Biopac Student Lab) was monitored, as were cardio-vascular and ventilation responses using a gas analyzer (Powercube-Ergo, Gansborn Medizine Electronic), the Borg's RPE scale was also measured. The protocol of the maximal test consisted of 6 minutes of warming up at 6km/h, followed by a graded exercise test on a treadmill with a constant slope of 1,5%. All values (VO2, VCO2, RER, VE, HR, RPE, Ts) were monitored at rest, during exercise and during 10 min of the recovery period. Data analysis included descriptives and One-Way ANOVA.

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Relation between Thermoregulation and VO2max in male endurance athletes

Results: VO2peak was 48,5 (5,3). All values significantly increased from basal until peak point. All variables decreased after their peak values during recovery period, except Ts which was 35,7 (1,1) and RER which was 1,1 (0,1); both were higher higher after 5'. ANOVA showed a major Ts variability (p=.044) correlated with VO2peak (p=.024) values.

Table 1: Thermoregulation variability related to aerobic power

	Evaluation phases					*(p< .05)			
VARIABLES	C1. BASAL (SD)	C2. WARMING UP (SD)	C3. END OF THE TEST (SD)	C4. RECOVERY 5' (SD)	C5. RECOVERY 10' (SD)	C1-2-3-4-5			
Heart Rate (bpm)	61.7 (8.1)	100.3 (10.1)	178.1 (10.7)	106.4 (915)	98.2 (12.8)	C1 - *C2, 3,4,5	C2 - *C3	C3 - *C4, 5	C4 - *C5
VO2 peak (ml·kg-1·min-1)	4.7 (0.7)	19.6 (3.5)	48.5 (5.4)	10.3 (2.4)	7.1 (2.4)	C1 - *C2,3, 4	C2 - *C3	C3 - *C4	
RER	0.7 (0.1)	0.8 (0.1)	1 (0.1)	1.1 (0.1)	0.9 (0.1)	C1 - *C2,3, 4	C2 - *C1,4		
Skin Temperature (°C)	33.8 (0.4)	34 (0.7)	35.7 (1.3)	35.7 (1.1)	34.9 (1.9)	C3 - *C 4,5			
Borg's Scale	6 (0)	7.8 (1.2)	19.7 (0.5)	9.1 (1.6)	6.6 (1)	C1 - *C2, 3,4,5	C2 - *C4	C3 - *C4, 5	C4 - *C5

Note: values are mean (Standard Deviation). C1-2-3-4-5 are the evaluation phases. Significance was considered at p< .05.

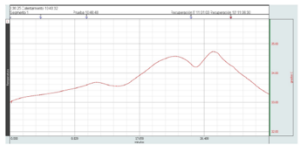

Figure 1. Ts evolution for running test

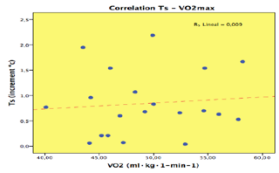

Figure 2. Correlation Ts - VO2max

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Relation between Thermoregulation and VO2max in male endurance athletes

Discussion: Results showed that there was a better aerobic power on subjects that had a better thermoregulatory variability of skin temperature during the test and in the recovery period. A higher thermoregulatory variability during treadmill test could determine the VO2peak and indicate a better autonomic nervous system response to endurance exercise. More research is required to determine how the thermoregulatory component could provide a better VO2peak.

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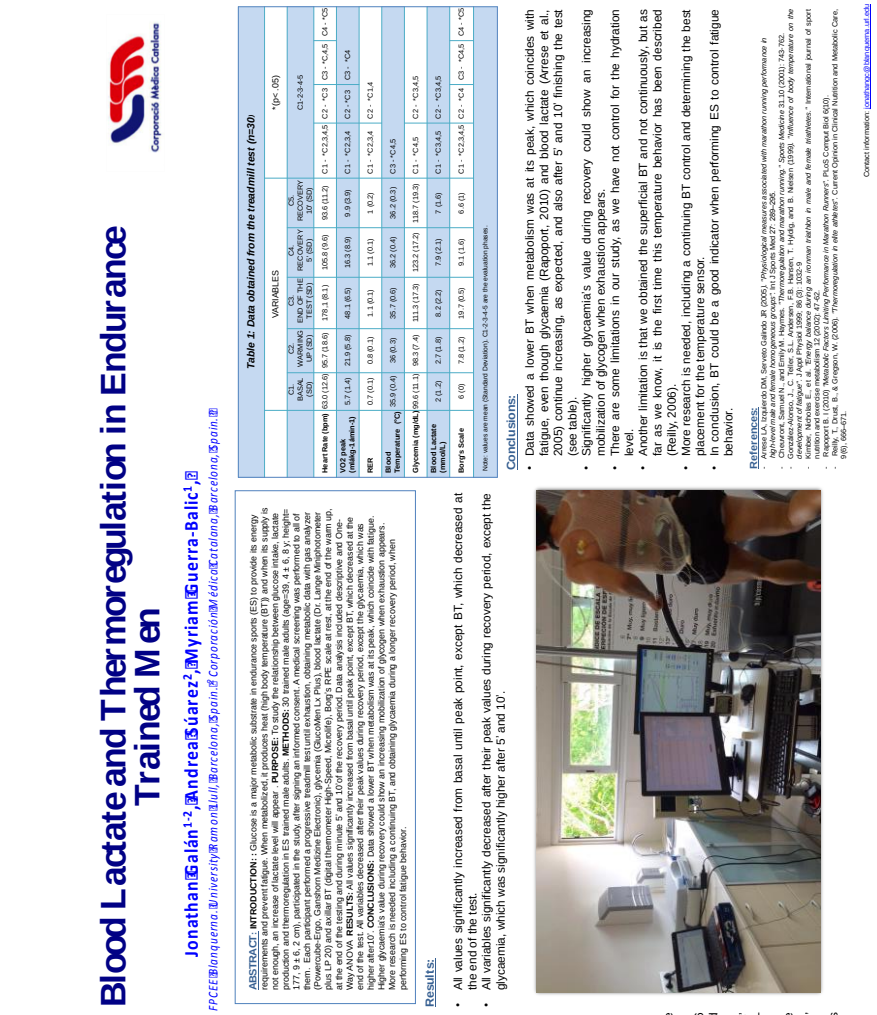
- **2016 (American College of Sports Medicine, ACSM) – Boston (USA)**



Glycaemia, Blood Lactate and Thermoregulation in Endurance Trained Men

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ABSTRACT: **INTRODUCTION:** Glucose is a major metabolic substrate in endurance sports (ES) to provide its energy requirements. When its supply is not enough, an increase of lactate level will appear. **PURPOSE:** To study the relationship between glycaemia, lactate production and thermoregulation in ES trained male adults. **METHODS:** 30 trained male adults (age=39, 4 ± 6, 8 y; height= 177, 9 ± 6, 2 cm) participated in the study after signing an informed consent. A medical screening was performed to all of them. Each participant performed a progressive treadmill test beginning walking at 6 km/h, increasing 1 km/h every 2 minutes, until exhaustion, obtaining metabolic data with gas analyzer (Powercube-Ergo, Ganshorn Medizine Electronic) and Borg's RPE scale. Blood lactate (Lactate Pro 2, Arkray) was also obtained (plus LP 20) and axillar BT (digital thermometer High-Speed, Microlife) at the end of the testing and during minute 5' and 10'of the recovery period. Data analysis included descriptive and One-Way ANOVA. **RESULTS:** All values significantly increased from basal until peak point, except BT, which decreased at the end of the test. All variables significantly decreased after their peak values during recovery period, except the glycaemia, which was significantly higher after10'. **CONCLUSIONS:** Data showed a lower BT when metabolism was at its peak, which coincide with fatigue. Higher glycaemia's value during recovery could show an increasing mobilization of glycogen when exhaustion appears. More research is needed including a continuing BT, and obtaining glycaemia during a longer recovery period, when performing ES to control fatigue behavior.

Introduction:

- Glucose is a major metabolic source in endurance sports (ES) to provide its energy requirements (Rapoport, 2010) and prevent fatigue (Gonzalez et al, 1999).
- When the glucose is metabolized, it produces heat and high body temperature (BT); and when its supply is not enough, an increase of lactate level will appear (Arrese et al., 2005).
- Previous studies about BT behavior when exercising showed that the metabolic variables could play a significant role in influencing the thermoregulatory answer (Kimber, 2002).
- Thermoregulation is a key to control the fatigue and regulate the correct working of body homeostasis process.

Purpose:

To study the relationship between glycaemia, lactate production and thermoregulation in ES trained male adults.

Subjects:

- 30 trained male adults (age=39, 4 ± 6, 8 y; height= 177, 9 ± 6, 2 cm and weight= 76,1 ± 5,9 kg), participated in the study, after signing an informed consent.
- A medical screening was performed to all of them including medical interview, general medical examination, body composition, isometric strength, flexibility, EKG at rest and during the treadmill test.

Materials & Methods:

- All the tests were performed at the same schedule time, in a room with a stable humidity level and a temperature of 23,5-24 ºC.
- Each participant performed the progressive treadmill test beginning walking at 6 km/h, increasing 1 km/h every 2 minutes, until exhaustion. The warming period was at 6km/h during 6 minutes. The treadmill slope was constant at 1,5%.
- At rest, at the end of the warm up, at the end of the testing and during minute 5' and 10'of the recovery period, metabolic data with gas analyzer (Powercube-Ergo, Ganshorn Medizine Electronic) were obtained (VO2 peak and RER).
- It was also obtained the glycaemia (GlucoMen Lx Plus), blood lactate (Dr. Lange Miniphotometer plus LP 20), axillar BT (digital thermometer High-Speed, Microlife) and Borg's RPE scale.
- Data analysis included descriptive of all variables and One- Way ANOVA was applied to study the differences between the testing phases.

Table 1: Data obtained from the treadmill test (n=30.

	VARIABLES						*(p< .05)
	C1. BASAL (SD)	C2. WARMING UP (SD)	C3. END OF THE TEST (SD)	C4. RECOVERY 5' (SD)	C5. RECOVERY 10' (SD)	C1-2-3-4-5	
Heart Rate (bpm)	63.0 (12.6)	95.7 (18.6)	178.1 (8.1)	105.8 (9.6)	93.6 (11.2)	C1 - *C2,3,4,5	C2 - *C3	C3 - *C4,5	C4 - *C5
VO2 peak (ml/kg-1.min-1)	5.7 (1.4)	21.9 (5.8)	48.3 (6.5)	16.3 (6.9)	9.9 (3.9)	C1 - *C2,3,4	C2 - *C3	C3 - *C4	
RER	0.7 (0.1)	0.8 (0.1)	1.1 (0.1)	1.1 (0.1)	1 (0.2)	C1 - *C2,3,4	C2 - *C1,4		
Rectal Temperature (ºC)	35.9 (0.4)	36 (0.3)	35.7 (0.6)	36.2 (0.4)	36.2 (0.3)	C23- *C4,5			
Glycemia (mg/dL)	90.6 (11.1)	98.3 (7.4)	111.3 (17.3)	123.2 (17.2)	118.7 (19.3)	C1 - *C4,5	C2 - *C3,4,5		
Blood Lactate (mmol/L)	2 (1.2)	2.7 (1.8)	8.2 (2.2)	7.9 (2.1)	7 (1.6)	C1 - *C3,4,5	C2 - *C3,4,5		
Borg's Scale	6 (0)	7.8 (1.2)	19.7 (0.5)	9.1 (1.6)	6.6 (1)	C1 - *C2,3,4,5	C2 - *C4	C3 - *C4,5	C4 - *C5

Note: values are mean (Standard Deviation). C1-2-3-4-5 are the evaluation phases.

Results:

- All values significantly increased from basal until peak point, except BT, which decreased at the end of the test.
- All variables significantly decreased after their peak values during recovery period, except the glycaemia, which was significantly higher after 5' and 10'.

Conclusions:

- Data showed a lower BT when metabolism was at its peak, which coincides with fatigue, even though glycaemia (Rapoport, 2010) and blood lactate (Arrese et al., 2005) continue increasing, as expected, and also after 5' and 10' finishing the test (see table).
- Significantly higher glycaemia's value during recovery could show an increasing mobilization of glycogen when exhaustion appears.
- There are some limitations in our study, as we have not control for the hydration level.
- Another limitation is that we obtained the superficial BT and not continuously, but as far as we know, it is the first time this temperature behavior has been described (Reilly, 2006).
- More research is needed, including a continuing BT control and determining the best placement for the temperature sensor.
- In conclusion, BT could be a good indicator when performing ES to control fatigue behavior.

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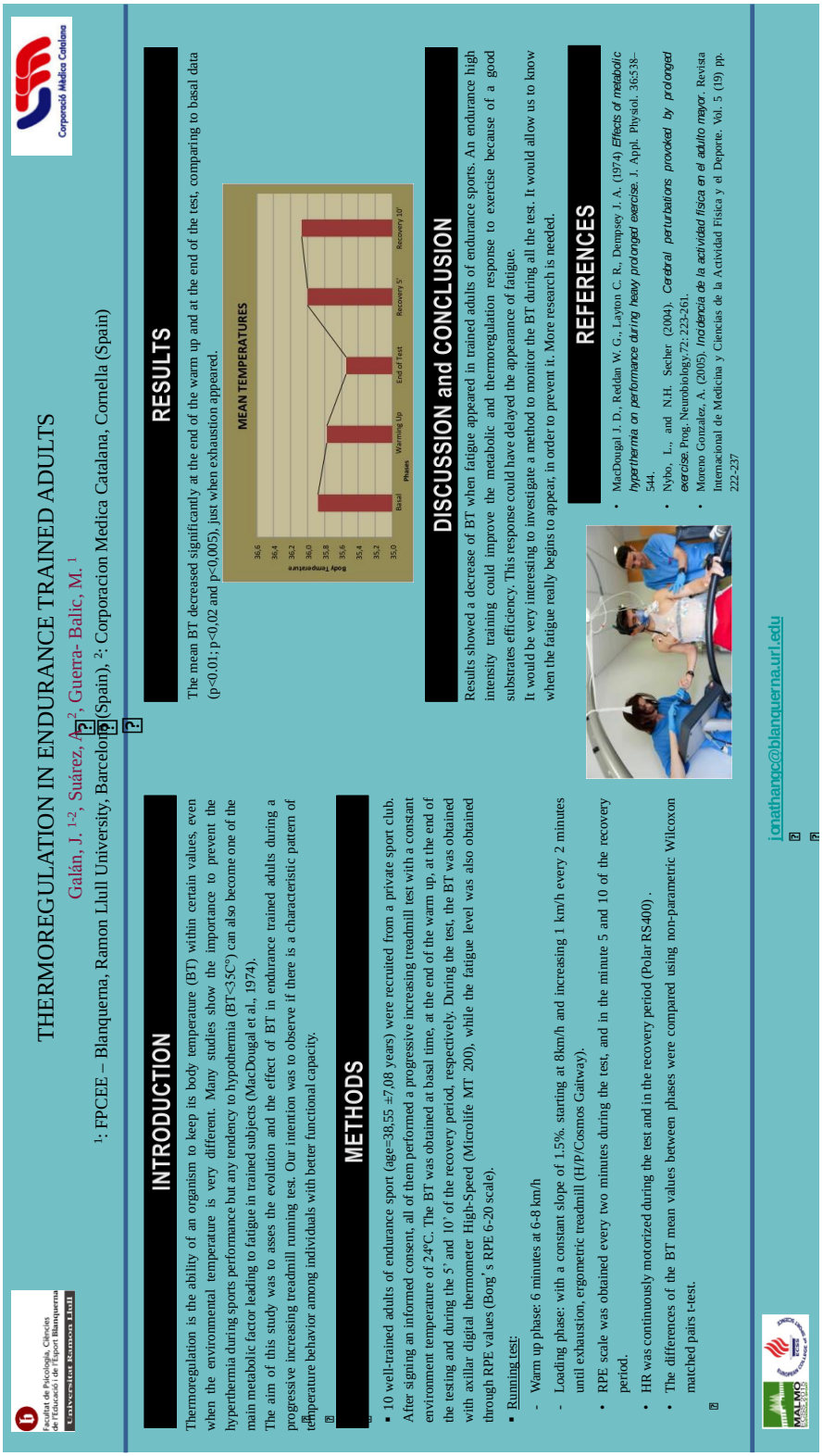
- **2015 (European College of Sports Science, ECSS) – Malmo (Norway)**



Facultat de Psicologia, Ciències de l'Educació i de l'Esport Blanquerna
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Corporació Mèdica Catalana

THERMOREGULATION IN ENDURANCE TRAINED ADULTS

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INTRODUCTION

Thermoregulation is the ability of an organism to keep its body temperature (BT) within certain values, even when the environmental temperature is very different. Many studies show the importance to prevent the hyperthermia during sports performance but any tendency to hypothermia (BT<35C°) can also become one of the main metabolic factor leading to fatigue in trained subjects (MacDougal et al., 1974).

The aim of this study was to asses the evolution and the effect of BT in endurance trained adults during a progressive increasing treadmill running test. Our intention was to observe if there is a characteristic pattern of temperature behavior among individuals with better functional capacity.

METHODS

- 10 well-trained adults of endurance sport (age=38,55 ±7,08 years) were recruited from a private sport club. After signing an informed consent, all of them performed a progressive increasing treadmill test with a constant environment temperature of 24ºC. The BT was obtained at basal time, at the end of the warm up, at the end of the testing and during the 5' and 10' of the recovery period, respectively. During the test, the BT was obtained with axillar digital thermometer High-Speed (Microlife MT 200), while the fatigue level was also obtained through RPE values (Borg´s RPE 6-20 scale).
- Running test:
  - Warm up phase: 6 minutes at 6-8 km/h
  - Loading phase: with a constant slope of 1.5%. starting at 8km/h and increasing 1 km/h every 2 minutes until exhaustion, ergometric treadmill (H/P/Cosmos Gaitway).
- RPE scale was obtained every two minutes during the test, and in the minute 5 and 10 of the recovery period.
- HR was continuously motorized during the test and in the recovery period (Polar RS400).
- The differences of the BT mean values between phases were compared using non-parametric Wilcoxon matched pairs t-test.

RESULTS

The mean BT decreased significantly at the end of the warm up and at the end of the test, comparing to basal data ($p<0.01$; $p<0.02$ and $p<0.005$), just when exhaustion appeared.

DISCUSSION and CONCLUSION

Results showed a decrease of BT when fatigue appeared in trained adults of endurance sports. An endurance high intensity training could improve the metabolic and thermoregulation response to exercise because of a good substrates efficiency. This response could have delayed the appearance of fatigue.

It would be very interesting to investigate a method to monitor the BT during all the test. It would allow us to know when the fatigue really begins to appear, in order to prevent it. More research is needed.

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Aquesta Tesi Doctoral ha estat defensada el dia ____ d_____ de 201__

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