UNIVERSITY OF NOVA GORICA
GRADUATE SCHOOL

DEHYDRATION AS ONE OF THE ENVIRONMENTALLY POTENTIATED HEALTH RISKS

MASTER´S THESIS

Jana Blatnik Mihovec

Mentor: dr. Petra Golja

Nova Gorica, 2008

ACKNOWLEDGEMENTS

First of all, I would like to thank my mentor dr. Petra Golja for giving me the opportunity to work on this interesting project. Her guidance, infinite patience and willingness to help were most significant and I could not have succeeded without it.

My thank goes to prof. dr. Igor Mekjavić from Jožef Stefan Institute, who provided the laboratory needed for the experimental part of the project. Thank goes also to doc. dr. Tatjana Tomazo-Ravnik from University of Ljubljana, Biotechnical Faculty, Department of Biology, for lending us the bioimpedance analyzer.

I would like to thank prof. Helena Jošt for having done the proof-reading work.

To my parents and Tanja: Thank you for being there for me, not just during this studying. I would not be here without you.

To my husband Dejan: Thank you for sharing your world and your life with me.

Last, but not least, I would like to thank all my friends for listening and sharing their worldly wisdom with me.

ABSTRACT

Growing and comprehensive evidence has been gathered over the last years suggesting that the climate of the Earth is changing as a consequence of human activities. A change in climate has been affecting and will continue to affect human performance, well being and health. The first effects of increased ambient temperature on human health have already been documented and include increased mortality rates during heat-waves. As dehydration has been proposed as a prominent cause for the reported increase in mortality during heat waves (EEA, 2005), it seems necessary to evaluate its detection; so far, no agreement has yet been reached on which of the existing methods could be used as standard methods for the detection of dehydration, and which of them are sensitive enough to detect small changes in hydration level. Secondly, the risk of dehydration during daily activities has to be assessed, as this risk will most likely increase in extreme weather conditions. The present study therefore compared five different methods for the detection of dehydration and assessed water replenishment capacity with drinking *ad libitum* during exercise in a moderate temperature environment.

Several measurements of hydration status, including changes in body mass, plasma volume, urine specific gravity, classical bioelectrical impedance parameters, and BIA-vector were performed on sixteen healthy young subjects before and after a three-hour walk in a moderately warm environment. The subjects were included either into a normohydrated group who ingested water *ad libitum* during the walk, or into a dehydrated group who was not allowed to drink during the experiment.

The results of the study demonstrated that if the measuring conditions are standardized, body mass measurements and urine specific gravity assessment are the most sensitive methods for the detection of dehydration. In contrast, the bioimpedance measurements (both, classical bioimpedance and BIA-vector) as well as plasma volume changes, as calculated from haemoglobin and hematocrit values, failed to detect dehydration. Furthermore, the results of the study suggest that in the normohydrated group only 49 % of the body mass lost during the walk was replaced, despite the fact that *ad-libitum* water ingestion was allowed. Nevertheless, the observed fluid loss remained below 2 % of body mass, which has been classified as a range of normal hydration (Sawka and Montain, 2000).

KEY WORDS: climate, global warming, health risks, dehydration, fluid replacement, urine specific gravity, plasma volume, bioelectrical impedance

POVZETEK

V zadnjih letih je zbranih vedno več obširnih dokazov, ki domnevajo, da se zemeljsko podnebje spreminja zaradi posledic človekove dejavnosti. Sprememba podnebja je in bo še naprej vplivala na človekovo zmogljivost, obstoj in zdravje. Prvi vplivi zvišanja okoljske temperature na človekovo zdravje so bili že dokazani in vključujejo povečano umrljivost v času toplotnih valov. Ker je dehidracija eden od pomembnih razlogov za povečanje umrljivosti v času toplotnih valov (EEA, 2005), je pomembno določiti najustreznejši način njenega določanja; do sedaj namreč še ni soglasja o tem, katera od obstoječih metod bi lahko bila standardna metoda za določitev dehidracije in katera metoda je dovolj občutljiva, da bi zaznala majhne spremembe v stopnji hidracije. Nadalje, oceniti bi bilo potrebno tveganje za nastanek dehidracije med dnevno aktivnostjo, kajti le-to se bo ob ekstremnih vremenskih pogojih najverjetneje stopnjevalo. Pričujoča naloga primerja pet različnih metod za določitev dehidracije in ocenjuje, kako učinkovito je nadomeščanje izgubljene tekočine s pitjem *ad libitum* med telesno aktivnostjo v zmerno toplem okolju.

V okviru naloge z naslovom Dehidracija kot zdravstveno tveganje zaradi okoljskih vplivov, je bilo izvedenih več različnih meritev za oceno dehidracije in sicer določanje sprememb v telesni masi, volumnu krvne plazme, specifični teži urina, klasična bioimpedanca in BIA vektor. Meritve so bile izvedene na šestnajstih mladih, zdravih preiskovancih in sicer pred in po triurni hoji v zmerno toplem okolju. Preiskovanci so bili razdeljeni v normalno hidrirano skupino, ki je med pohodom lahko pila vodo *ad libitum*, ali pa v dehidrirano skupino, ki med poskusim ni uživala tekočine.

Rezultati raziskave so pokazali, da sta, če so pogoji standardizirani, merjenje telesne mase in specifične teže urina najbolj občutljivi metodi za določanje dehidracije. Nasprotno, bioimpedanca (obe, klasična in BIA vektor) in sprememba volumna krvne plazme, izračunana iz vrednosti hemoglobina in hematokrita, nista zaznali dehidracije. Nadalje, rezultati raziskave so pokazali, da so preiskovanci v normalno hidrirani skupini nadomestili le 49 % izgubljene telesne mase, kljub temu, da so vodo lahko zauživali *ad libitum.* Poudariti pa je treba, da je bila opažena izguba tekočine, kljub na videz neustreznemu nadomeščanju tekočine, manjša od 2 % telesne mase, kar označujemo kot normalno hidracijsko stanje (Sawka and Montain, 2000).

KLJUČNE BESEDE: podnebje, globalno ogrevanje, zdravstveno tveganje, dehidracija, nadomeščanje tekočine, specifična teža urina, volumen plazme, bioimpedanca

I. TABLE OF CONTENTS

II. LIST OF FIGURES

III. LIST OF TABLES

IV. LIST OF APPENDICES

ABBREVIATIONS AND SYMBOLS

EEA	European Environmental Agency
IPCC	Intergovernmental Panel on Climate Change
UNEP	United Nations Environmental Program
UNFCCC	United Nations Framework Convention on Climate Change
WHO	World Health Organization
WMO	World Meteorological Organization

1. INTRODUCTION

Global warming is one of the most serious challenges the humanity is facing today. Increasing evidence demonstrates that atmospheric levels of carbon dioxide are rising and that they are the main cause for global warming (Keatinge, 2003). Many high quality reports and journal articles, which have been published in the recent years (Houghton, 2001; IPCC, 2001; EEA, 2004, IPCC, 2007b), agree that it is the emission of greenhouse gases which is causing, and will continue to cause most of the recently observed climatic changes, which result in global warming.

Any change in ambient temperature undoubtedly affects human performance, well being and health and the first effects of increased ambient temperature on human health have already been documented. An intergovernmental body International panel on climate change (IPCC), the recipient of 2007 Nobel peace prize, for example, focused on heat-related mortality in Europe (IPCC, 2007b). They, as well as others (Donaldson et al., 2001), demonstrated a clear association between ambient temperature during summer heat waves and number of heat-related casualties.

Furthermore, the IPCC report (2007a) expects that heat waves related to global warming, which are expected in the future, will contribute to increased rates of heat-related casualties around the world, as most cities that currently experience heat waves are likely to be exposed to increased number, intensity, and duration of heat waves in the future. The balance of positive and negative impacts of climate change on human health will inevitably be established; the effects will vary from one location to another, and will be changing over time as the ambient temperatures continue to rise. It is expected that the negative impacts on human health will be experienced primarily by the populations, which are the most vulnerable to increases in ambient temperature; these are the elderly, children, the poorest, people suffering from predisposing chronic medical conditions, overweight people, and people who work and perform endurance activities in a climatically stressful environment (Barrow et al., 1998; Koppe et al., 2004).

In order to prevent the negative effects of increasing environmental temperatures on human health, we need to detect early sings of heat related health problems. One of the most common

heat-related problems is undoubtedly dehydration, which will be the focus of the present thesis. In order to detect dehydration soon enough to prevent several of its negative effects, we need to identify, which are the most appropriate methods, which are able to detect the first signs of dehydration. An overview and comparison of the methods used to assess dehydration in a group of experimental subjects will present the core of the present thesis.

In the first part of the thesis, the main relations between man and the environment, with a particular emphasis on the effects of climate change on human health and well being will be described. Furthermore, the maintenance of stable deep body temperature and heat related health problems, with a particular emphasis on dehydration will be presented. The experimental part of the thesis will investigate the questions, which are the most appropriate methods for the detection of dehydration. Different methods will be compared on a sample of subjects and their advantages and disadvantages will be described.

2. LITERATURE REVIEW

2.1 Climate change

The needs of a human organism for food, fresh water, clean air and relative climatic constancy are basic and unalterable. Favourable climatic conditions are essential to human well-being and especially to human health, which, according to World health organization, is a state of complete physical, mental and social well-being, and not merely the absence of disease or infirmity (WHO, 1984). Both, climatic factors and living organisms are already extremely complex systems on their own and it is therefore even more difficult to assess and understand various interactions between them (Cegnar, 2004). In the last decades, scientific community has focused extensively on the potential effects of climate change on human survival and well-being. Despite the fact that certain progress has been made with respect to these topics in the last years, several questions still remain unanswered and the issues regarding the effects of climate change on human health and well-being remain largely unresolved.

The interactions between atmospheric processes and living organisms, plants, animals and humans, are the focus of biometeorology, which is an interdisciplinary scientific discipline (Kajfež-Bogataj, 2005). When assessing the impacts of the environmental factors on human health and well being, biometeorology tries to account for as many parameters as possible, but as this is usually not feasible, research activities focus on individual environmental variables. In the last decade, especially after the year 2003, when a severe heat-wave hit Europe and caused significant mortality particularly amongst the elderly population, the focus of biometeorology has shifted towards the investigation of climatic change and its effects on human health. Thus, extensive research activities have been performed on the effects of human exposure to high environmental temperatures, high air humidity and heat radiation (Kajfež-Bogataj, 2005).

Climate change is one of the greatest environmental, social and economic threats to the planet, as recognized by several international agencies (WMO, WHO, UNEP). Reports by WHO warn that a global climate change could affect human health in many different ways (Kalkstein & Smoyer, 1993), some of them are presented in Figure 1. According to the expectations (Cegnar, 2005), the size and nature of the impacts of a climate change on human health would vary according to a geographical region (climate change and the associated

global warming in particular is expected to have the greatest amplitudes over the land areas), vulnerability of population groups, the size and duration of exposure to challenging climatic conditions, and society's ability to adapt to the change. Several direct and indirect threatening environmental impacts on human health have already been described and include: changes in environmental temperature, UV radiation, quality of air, as well as the occurrence of extreme weather, outbreaks of infectious diseases, migrations, changes in flora and fauna, changes in food and drinking water quality, etc. (Cegnar, 2005).

New and stronger evidence suggests that most of the warming, which has been observed in the last 50 years, is attributable to either direct or indirect human activities (Houghton et al., 2001; IPCC, 2001). Data demonstrate that the amount of greenhouse gases in the atmosphere has increased substantially from the year 1750, and that it is still increasing at an unprecedented rate of 0.4% per year on average (Houghton et al., 2001), which traps more and more heat in the lower atmosphere. Since 1750, the amount of CH_4 has increased by 151%, N_2O by 17%, and O_3 by 36% (Houghton et al., 2001; EEA, 2004). Furthermore, global emissions of CO_2, CH_4 and N_2O, have increased by 70% between 1970 and 2004 (IPPC, 2007). In the same time period, CO_2 emissions have grown by about 80%, and 28% of this increase has been observed between 1990 and 2004 (IPPC, 2007c). The last report by IPCC presented in 2007 (IPCC, 2007) has thus established with a great certainty that it is the human activities, which are responsible for the observed global warming and the associated climate change. Furthermore, many scientific journals (Mitchell et al., 1995; Houghton et al., 2001; McCarthy et al., 2001) published between 1993 and 2006 confirm the previously published consensus position of the IPCC (2001), stating that the emissions of greenhouse gases have caused, and will continue to cause, most of the global warming observed in the last decades.

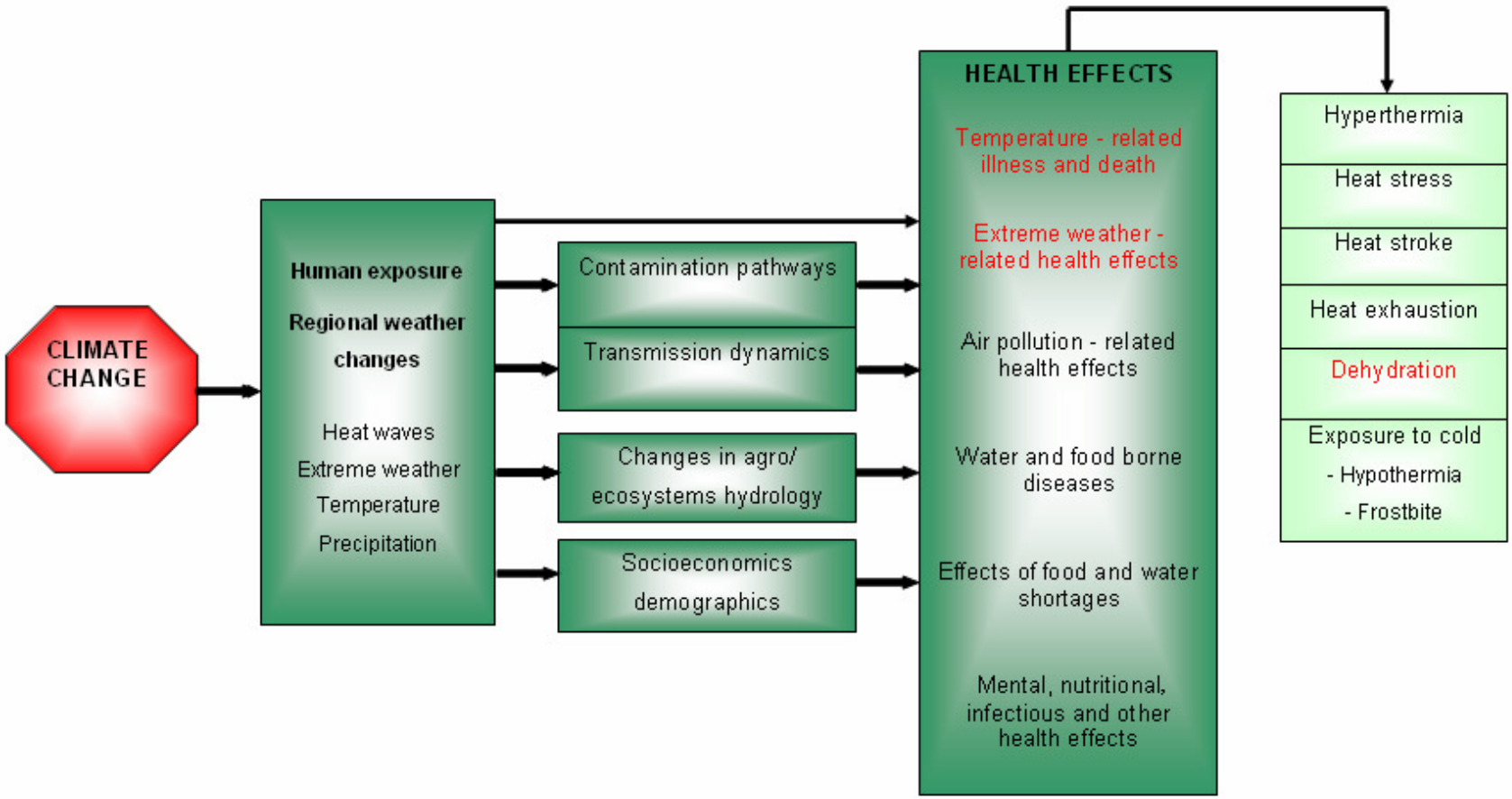

Figure 1: *Pathways by which climate change may affect human health (Modified from: WHO, 2003; WMO, 2003; UNEP, 2003).*

The generally accepted main cause for the observed climate change is the greenhouse effect. The largest global greenhouse gas emissions come from energy supply sector, industry, land use, land use change, and forestry (IPPC, 2007c); the last three terms are used to describe the common emissions of greenhouse gasses from deforestation, biomass loss because of burning and logging, decay of peat and peat fires.

The greenhouse effect maintains the average surface temperature of the planet suitable for human survival. Namely, about two thirds of the solar energy reaching the Earth is absorbed by the Earth's surface. Some of the heat radiates back to the atmosphere, is trapped by greenhouse gases, such as CO_2, CH_4 and N_2O, and is then re-emitted in all directions. The greenhouse gases in the atmosphere thus allow the incoming infrared radiation to pass through the Earth's atmosphere, but at the same time prevent most of the outgoing infrared radiation from the surface and lower parts of the atmosphere from escaping into the outer space (Figure 2).

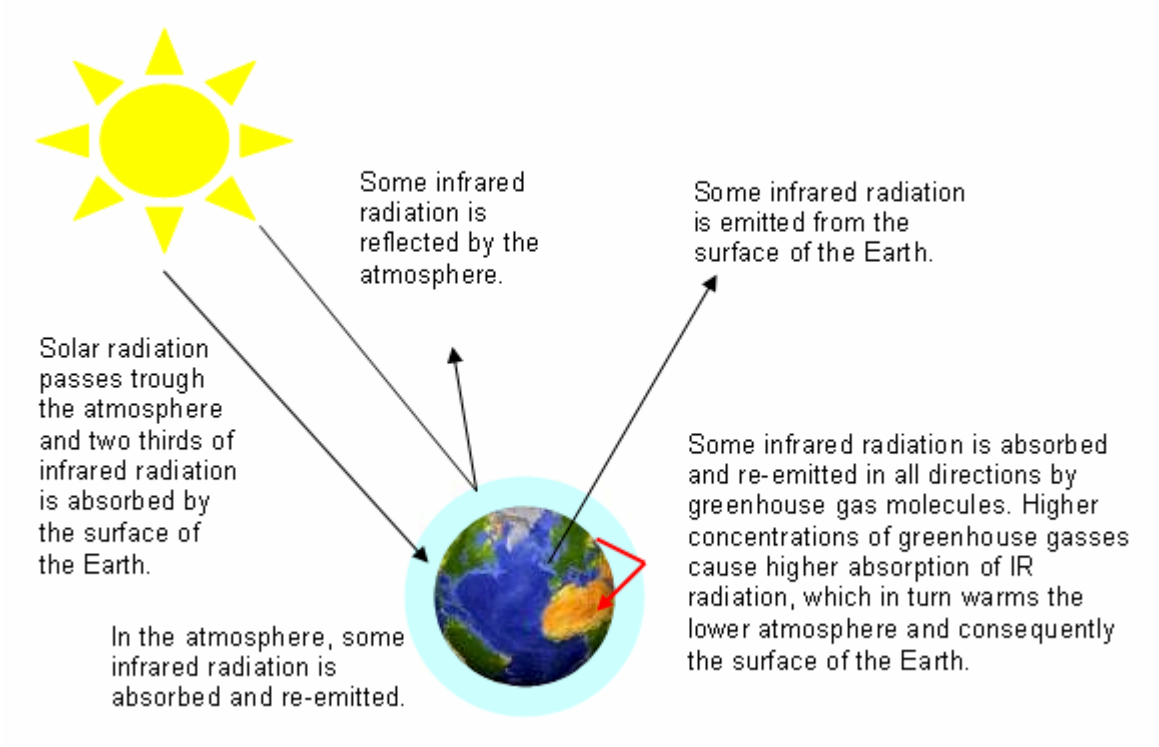

Figure 2: *The greenhouse effect (modified from US EPA, 2001).*

Concomitant with an increase in the amount of greenhouse gasses in the atmosphere, the average (SD) surface temperature has increased by 0.6 (0.2)°C over the last century (Houghton et al., 2001), and approximately two-thirds of this warming has occurred since 1961 (McCarthy et al., 2001). Since the year 1979, both satellite and weather balloon measurements demonstrate that the global average temperature of the lowest 8 kilometres of the atmosphere has changed by +0.05 (0.1)°C per decade, and that the global average surface temperature has increased significantly by +0.15 (0.1)°C per decade (Houghton et al., 2001). The warming is expected to continue (Figure 3) and the average surface temperature of the Earth is expected to rise from 14,5°C, as measured at the end of the 20th century, to 16°C in the year 2050, and to 17°C at the end of 21st century (Carter et al, 2000).

It is reasonable to assume that such drastic changes in average surface temperature of the Earth and consequently in climate will also affect human survival, performance, well-being and health. Therefore, the links between climate change and human health are being increasingly identified. The observed changes in ambient temperature have already been associated with several negative effects on human health in recent decades (Hughes, 2000), with heat wave related health problems being the most prominent.

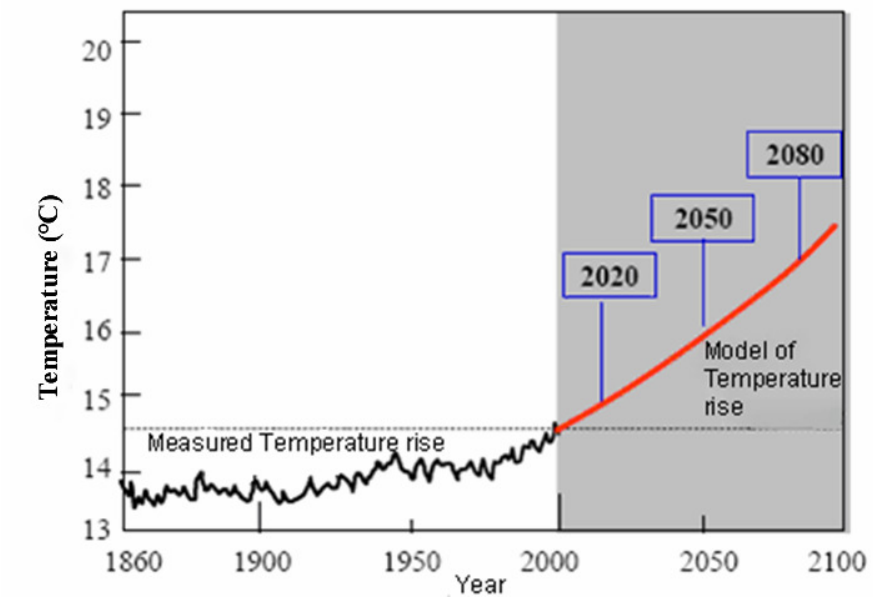

Figure 3: *A prediction of changes in average surface temperature of the Earth (modified from Kajfež-Bogataj, 2005).*

Heat waves have been identified as the most prominent cause of weather-related human mortality in big cities with high population density (Alberdi et al., 1998; Davis et al., 2003). Heat waves usually occur in synoptic situations with pronounced slow air mass movement, which leads to an intensive and prolonged heat stress (Koppe et al., 2004). Namely, in summer, buildings in a city detain heat and emit it even during the night - the flow of fresh air into the city is hindered and the city is therefore referred to as a heat island. The most prominent characteristic of a heat island is that environmental temperatures in the city centre remain very high until the late afternoon, with temperatures being a few degrees lower in suburban and rural parts (Figure 4).

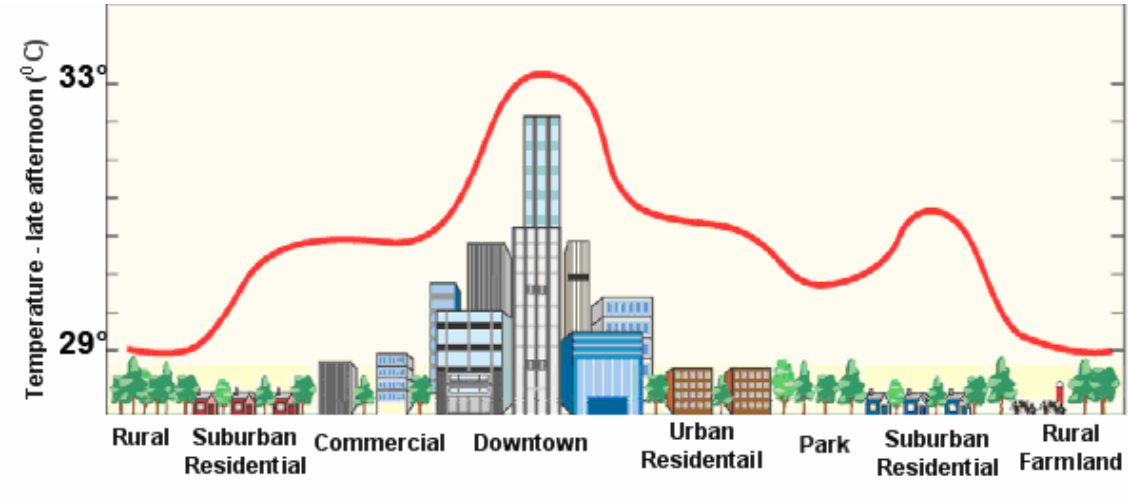

Figure 4: *Sketch of an urban heat island profile (modified from Oke, 1997). In the city centre air temperature remains very high until the late afternoon in summer or during heat waves, with temperatures being a few degrees lower in suburban and rural parts.*

In the year 2003, when one of the warmest summers in Europe was recorded, high human mortality was observed primarily in the cities, which had the attributes of a heat island, as the latter further aggravated the existing unfavourable weather circumstances (Arnfield, 2003). For example, in the beginning of August 2003, environmental temperatures in Paris (France), were increased considerably above the average values for this time of the year. Maximum daily temperatures reached up to 37°C. Concomitant with the observed high environmental temperatures, a 60 % increase in mortality (Pirad et al., 2005), as compared to the average daily mortality for the same period in the years 2000 and 2002 was recorded. The recorded number

of excess deaths followed the same pattern as the increase in environmental temperatures (Pirad et al., 2005). As presented in Figure 5, such association has also been described by other authors (Donaldson et al., 2001) at different locations and time periods.

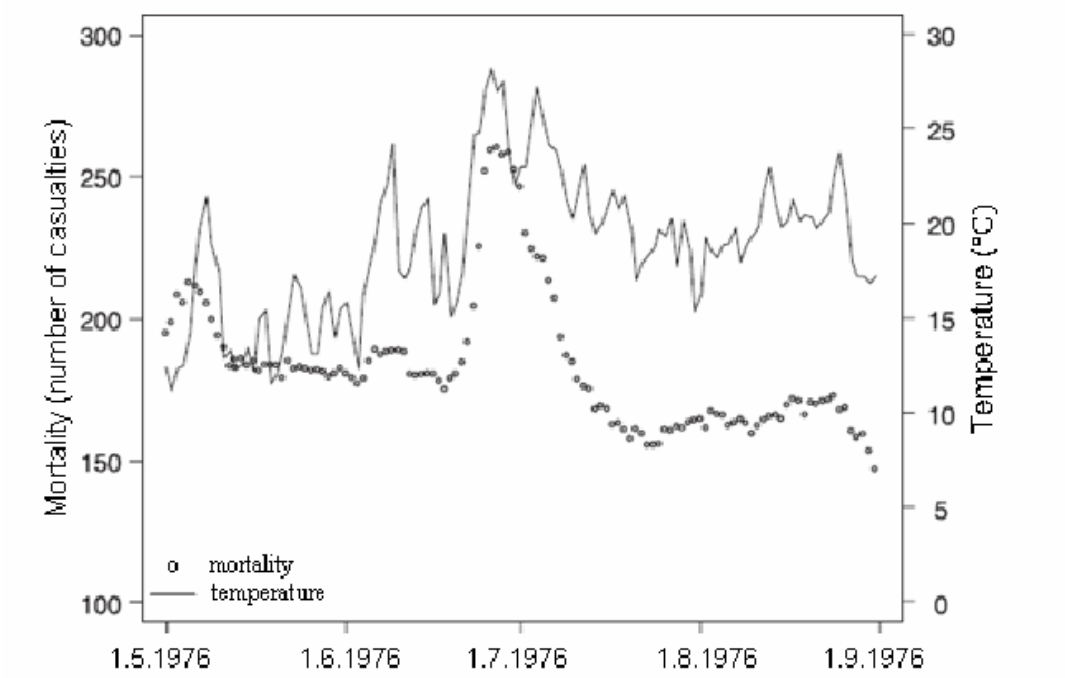

Figure 5: *The observed daily mortality during 1976 heat-wave in London (Modified from: Donaldson et al., 2001).*

During the 2003 heat wave, 35 000 heat-induced deaths in Europe were registered, and the majority of them have been attributed to both, cardiovascular problems and dehydration (EEA, 2005); the latter was especially frequent with elderly people (Martinez-Nevarro et al., 2004; EEA, 2005). Some reports (Larsen, 2006) suggest, that the consequences of the 2003 heat wave were even more drastic, with more than 52 000 people estimated to die as a result of prolonged heat exposure.

As presented above, the effects of environmental conditions on human health and even survival are undoubtedly prominent. Therefore, the second part of the literature review will focus on the physiological consequences of exposure to a warm environment.

2.2 Environmental impacts on human health

Humans are homoeothermic beings; we use physiological mechanisms of temperature regulation to maintain body temperature stable in various environmental conditions. The term temperature regulation or thermoregulation is used to describe processes related to the maintenance of stable deep body temperature. The mechanisms, which maintain deep body temperature stable, are regulated by a special region in the brain, the hypothalamus. The hypothalamus constantly balances the heat production and passive heat gain, with heat loss, which keeps the deep body temperature within an optimal range.

The body is thermally divided into a warmer internal core and a cooler outer shell, periphery (Figure 6). The body core comprises all vital organs inside the head and trunk, and is kept at approximately 37ºC (Eisman, 1972; Campbell, 1987; Brück, 1989, Purdy, 2000; King, 2004). The exact resting core temperature depends on an individual's metabolic rate; the higher it is, the higher is the resting body core temperature, and vice versa. Body core temperature also depends on the time of the day, level of physical activity, or emotional state. The temperature of the outer shell is to a greater extent affected by the environment, and is not regulated within as narrow limits, as body core temperature.

Thermoregulation is the ability of a person (animal) to keep its body temperature within certain boundaries, even when the temperature of the surrounding environment is very different from the body core temperature. Thermoregulation is thus a homeostatic mechanism; it maintains a dynamic state of stability between the internal and external environment.

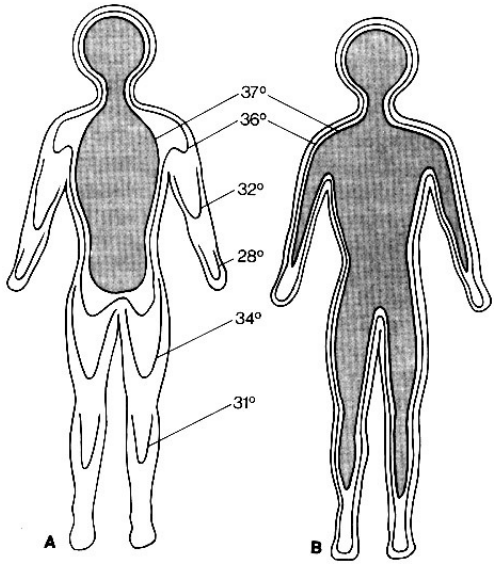

Figure 6: *The distribution of temperatures (^{o}C) within the body and the division of the body into thermal core and outer shell, thermal periphery, during exposure to (A) cold and (B) warm environment (modified from Brück, 1989).*

Exposure to a warm environment increases the strain on the thermoregulatory system. If the strain is too big to cope with, the thermoregulatory system is unable to maintain core temperature within the normal limits and a condition known as hyperthermia (Glossary of terms for thermal physiology, 2001), denoted by an increase in body core temperature, can occur. In a cold environment, the opposite condition can be induced, with body core temperature decreasing below the normal levels; this condition is referred to as hypothermia (Glossary of terms for thermal physiology, 2001).

2.3 Temperature regulation and heat related health problems

Exposure to a warm environment is not the only source, which increases core temperature, another powerful drive for core temperature changes is muscular activity, as approximately 75% of the energy produced during the activity is released in a form of heat (Ferguson, 2002). Thus, during exercise, when the metabolic activity is increased, the metabolic heat production is enhanced and the heat accumulates in the tissues. In a cold environment, the production of heat is favourable as it prevents hypothermia, but in a warm environment heat must be efficiently dissipated to prevent hyperthermia. Hyperthermia can be a serious health

risk condition, as it can result in heat stroke or other heat-related illnesses (Koppe et al., 2004). If, during exercise, a person was also exposed to a warm environment, the body core temperature would rise quickly, if there were not efficient mechanisms available to counteract the accumulation of heat in the body. Efficient heat dissipation thus decreases the risks of heat-related illnesses and mortality in a warm environment.

Dissipation of excess heat from the body to the environment occurs by four different heat exchange pathways: conduction, convection, electro-magnetic radiation and evaporation (Ferguson, 2002; King, 2004) (Figure 7).

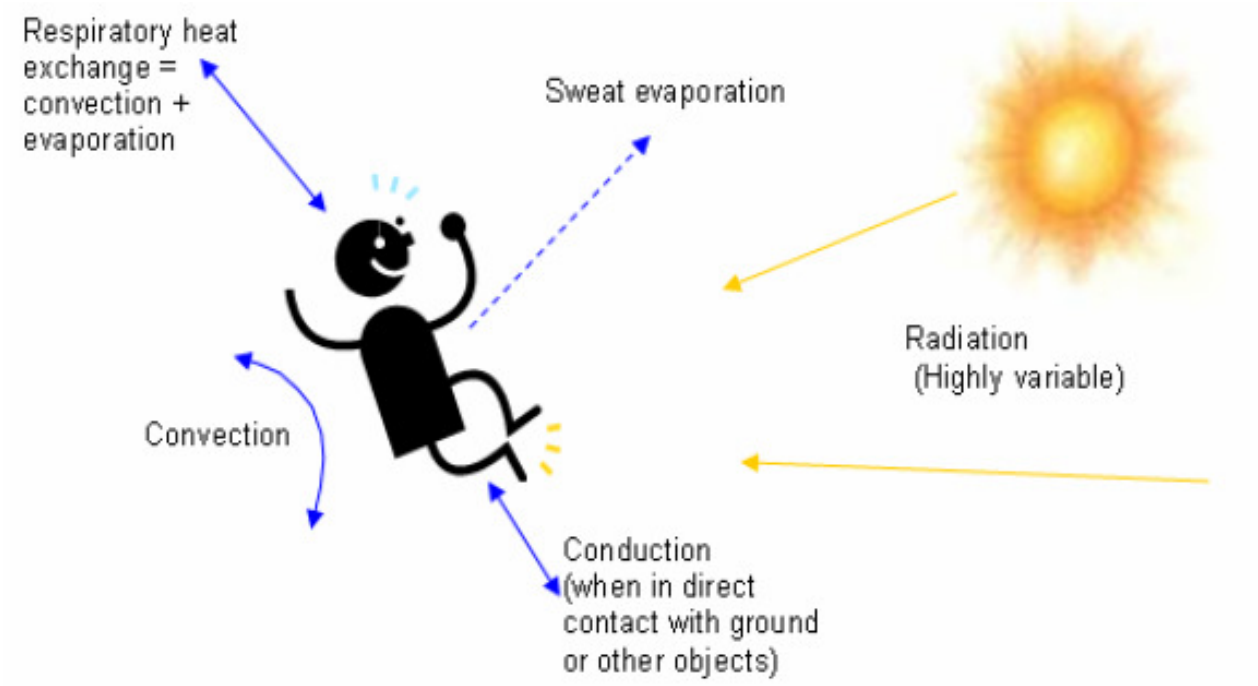

Figure 7: *Heat exchange pathways in humans (modified from Koppe, 2004).*

Heat exchange pathways determine the amount of heat stored or dissipated from the body according to a heat balance equation (Glossary of terms for thermal physiology, 2001) (Equation 1):

$$S = M - W - E - C - K - R \qquad \text{Equation 1}$$

where: S = storage of body heat [W/kg];

M = metabolic rate [W/kg];

W = work rate [W/kg];

E = evaporation - a transfer of heat by sweat or water evaporating from the skin [W/kg];

C = convection - a transfer of heat by movement of medium molecules (usually air or water) from warm to cool areas [W/kg];

K = conduction - a transfer of heat by direct contact to objects of a different temperature [W/kg];

R = radiation - a transfer of heat by electromagnetic radiation [W/kg].

Heat exchange between the body and the environment is usually regulated by adjusting skin blood flow. The flow of blood to the skin transports heat from the body core and muscles to the skin, where it is dissipated by convection. Most often, this mechanism is sufficient to maintain the core temperature stable. In an environment, where air temperature increases, the thermal gradients between the skin and the environment which are required for heat dissipation decrease. Consequently, the heat loss from the body by convection diminishes. When air temperature approaches the skin temperature, heat loss by convection approaches zero, and heat may even be gained, when air temperature rises above the skin temperature; this in turn increases the deep body temperature (Koppe et al., 2004). In a thermally uniform warm environment with no significant radiation sources or sinks, it is only the evaporation of sweat from the skin surface, which can efficiently dissipate heat from the body. The efficiency of evaporation is obvious if we know that the evaporation of 1 litre of sweat can dissipate 580 kcal (2.5 MJ) of heat (McArdle et al, 2001). Evaporation is thus the far most efficient mechanism for maintaining deep body temperature stable.

During heat waves, for example, the proper functioning of the thermoregulatory system is essential for the well being of humans, as the exposure to extreme climatic conditions presents a high physiological strain. If the thermoregulatory system cannot

match the thermoregulatory demands, this increases the body core temperature and may eventually cause heat-related illnesses (Koppe et al., 2004). Heat-related illnesses are more common in the summer, but can also occur in moderate conditions when associated with some environmental factors, such as high humidity, which prevents the efficient evaporation from the skin and thus reduces the efficiency of heat dissipation. The vast majority of heat-related illnesses result from the exposure to a hot environment, but they can be also induced by exercise, especially when the latter is performed in a warm environment (Barrow et al., 1998; Koppe et al., 2004).

Heat-related illnesses can be classified in different groups according to their symptoms, which range from mild weakness, dizziness and fatigue, to cramps, syncope, exhaustion and multisystem complications, including coma and death:

heat rash occurs when the sweat ducts to the skin become blocked, swollen, and cause discomfort, and itching;

heat cramps occur in muscles during or following exercise, because sweating causes the body to lose water, salt and other minerals (electrolytes), which can induce cramps;

heat oedema occurs because of the accumulation of body fluids in the legs and hands after sitting or standing for a prolonged period of time in a hot environment;

heat syncope (fainting) is a consequence of (too) low blood pressure, which is decreased because heat exposure causes the blood vessels to expand, and consequently body fluids accumulate in the lower lying parts of the body because of gravity;

heat exhaustion is a severe general fatigue usually caused by dehydration and is the most common heat illness (Kramer et al., 1989; Knochel, 1996);

heat stroke (sun stroke) occurs when the capacity of the thermoregulatory system is exceeded and body core temperature continues to rise. It is a medical emergency and even with immediate treatment can be life-threatening or can result in serious long-term health problems.

One of the phenomena, which is closely related to the heat-related illnesses in a warm environment, is the loss of water from the body due to an increased rate of sweating, i.e. dehydration. Because sweat evaporation increases heat loss from the

body, an increase in sweating results in a more effective deep body temperature regulation. At the same time, however, an increase of sweating also increases the water loss and thus the risk of dehydration. With an increased level of dehydration, the intensity of sweating decreases in order to maintain body water stores, however a reduced level of sweating due to dehydration decreases the capability of the thermoregulatory system to maintain core temperature stable. This, in turn, increases the risk of hyperthermia and heat–related illnesses (Wyndham and Strydom, 1969, as cited by Noakes, 2003b).

It has been demonstrated that dehydration of more than 3 percent of body mass is an important risk factor in heat-related illnesses (Hassanein et al., 1992); if the lost fluid is not restored, the risk of heat-related illness increases. It is thus obvious that one of the preventions against heat-related illnesses should include drinking of a suitable amount of fluids before, during and after exercise and/or the exposure to a warm environment (Barrow et al., 1998), as the efficient fluid replacement can prevent dehydration and enable efficient temperature regulation by sweat evaporation.

2.4. Dehydration and the maintenance of euhydrated state

In humans, water is the largest component of body mass. Body water typically accounts for approximately 60 - 70 % of body mass in males, and 50 - 55 % in females (Oppliger, 2002). Body water can be partitioned between intracellular and extracellular compartments, which contain 60 % and 40 % of the total body water, respectively (Wang et al, 1992, as cited by Oppliger, 2002; Sawka et al., 2005). Thus, a 70 kg man is composed of approximately 42 litres of water, of which 25 litres is intracellular, and 17 litres extracellular water. Extracellular water is further divided between the compartments (Bear, 1996), so that approximately 3 litres are located in the intravascular compartment (i.e. in plasma), 1 litre in the transcellular space, 8 litres in the interstitial fluid, and the rest of approximately 5 litres in bone and dense connective tissue (Figure 8).

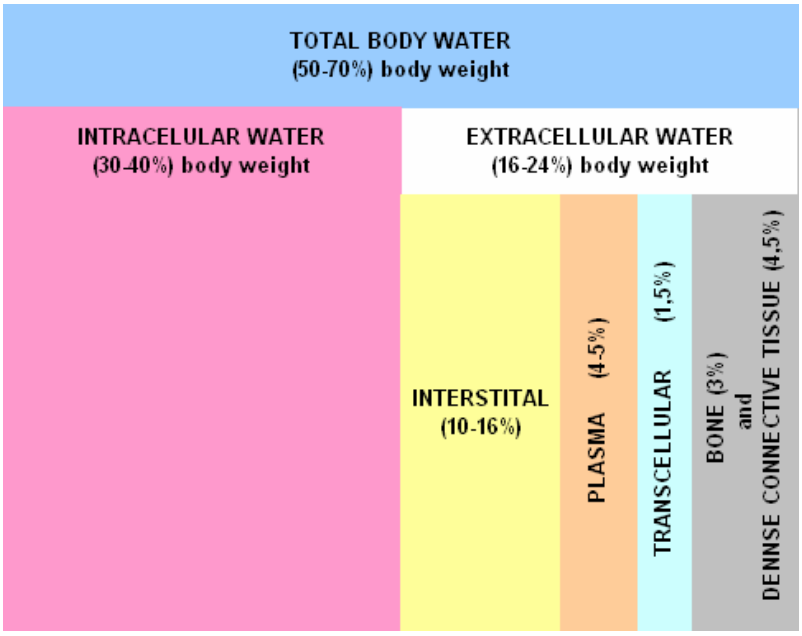

Figure 8: *The approximate size of body water compartments in a 70 kg adult (Modified from Bear, 1996; Krane, 1996; and Lippincott, 2005).*

A state of normal body water content has been defined as euhydration. This state has not been described as a specific optimal point, but rather as a sinusoidal wave that indicates normal, daily changes in optimal water content (Greenleaf, 1992). This definition is primarily theoretical in nature, and often no measurable criteria are mentioned for the detection of eu- and dehydration, primarily because dehydration is hard to define with a set of specific clinical symptoms (Weinberg and Minaker, 1995), despite the fact that several different scales relating dehydration levels and the corresponding medical symptoms have been established (Table 1). In the field of sports medicine, the level of dehydration is usually defined by the amount of body mass lost (usually by exercise) during a daily cycle or a specific activity. An athlete who loses 3 % of his/her body mass is thus considered 3 % dehydrated (Oppliger, 2002).

The level of (de)hydration depends on both, fluid loss and fluid uptake. The magnitude of fluid loss depends primarily on the intensity of sweating and is related to:

- the environmental conditions, such as ambient temperature, relative humidity, air motion and solar load, which affect heat exchange pathways;
- type of physical activity, its intensity and duration; as well as on

- the choice of clothing (Sawka et al., 2005).

Fluid uptake, on the other hand, is a voluntary activity (Bass and Inge, 2001).

The maintenance of euhydrated state during exercise in a hot environment remains a matter of extensive scientific debate. It has been proposed that a substantial increase of fluid intake is needed to maintain euhydration when ambient temperature rises above 25°C (Casa, 1999), however such recommendations can not simply be generalised because of a variety of conditions which affect the amount of fluid that is being lost from the body.

Furthermore, many different open questions on when people need to consume fluids (i.e. before, during or after exercise in a moderate to warm environment) to prevent dehydration remain unanswered, primarily because environmental factors play such an important role in the process of (un)successful rehydration.

Table 1: *Symptoms of dehydration with respect to the percentage of body mass loss (Koppe, 2004).*

Degree of dehydration	Fluid loss for a 70-kg person (litres)	Symptoms
2 %	1.4	thirst
4 %	2.8	the above and dry mouth
6 %	4.2	all the above, increased heart rate
8 %	5.6	all the above, swollen tongue, difficulties in speech, reduced mental and physical performance
12 %	8.4	all the above and recovery only after intravenous fluid administration
14 %	9.8	death

Last, but not least, the scientific debate has focused on the key factors that may provide a reliable sensory input to the central nervous system in order to counteract excessive fluid loss. Namely, some authors suggest that the feeling of thirst, which is initiated during the prolonged exercise or warm exposure, is not sufficient to maintain normal hydration, because the thirst is supposed to be elicited by losses of 1% to 2% of body mass, which are already classified as dehydration (Casa, 1999).

According to such opinion, a physically active person exercising in a warm environment should initiate drinking before the actual feeling of thirst is induced, so as to be able to prevent dehydration (Convertino et al., 1996). In contrast, other authors reported that excessive intake of liquid is rather harmful than advantageous (Noakes, 2003a), especially, when sodium lost by sweating is not replenished. Namely, when sodium loss by sweating is combined with an increased water intake, a potentially lethal hyponatremic condition may be induced (Noakes, 2003b).

The right method for the maintenance of optimal euhydrated state during physical activity in a warm environment therefore still remains elusive.

2.5. The assessment of hydration status

If one considers the consequences of climate change and global warming, it becomes evident that in the future humans will be more often exposed to extreme weather conditions, with all the adjoining heat-related health risks. As dehydration has been proposed as a prominent cause for the reported increases in mortality during heat waves (EEA, 2005), it seems necessary to evaluate, what countermeasures one can provide to avoid it. Secondly, it also seems reasonable to try to assess the risk of dehydration during daily activities, as this risk will most likely increase in extreme weather conditions.

As extreme levels of dehydration are usually not experienced during everyday life, the method used for the assessment of dehydration should be sensitive enough to detect small changes in hydration level. So far, several methods for the assessment of hydration status have been described in literature, but, rather surprisingly, no agreement has yet been reached on which one of these methods should be used as the standard method for the detection of dehydration. The most common methods for the detection of dehydration include: monitoring body mass changes, measuring changes in different blood parameters, particularly changes in plasma volume, and determining the changes in urine specific gravity. Furthermore, the use of new methods for the assessment of hydration status, such as various bioelectrical

impedance measurements, has also been proposed by some authors (Piccoli et al. 1994), but these methods have so far not been subject to extensive evaluation.

The methods for the assessment of hydration status, both classical and new, which were used in the present study, are shortly presented below.

2.5.1 Mass measurements

To assess dehydration induced by a certain activity or environmental exposure, body mass is measured before and after a certain activity/exposure. To provide reliable measurements of body mass, it is necessary that a certified scale is used for the measurements and that the person wears identical, preferably minimal clothing during all measurements.

2.5.2 Assessment of plasma volume

Fluid loss can also be assessed by monitoring relative changes in plasma volume. The latter are assessed by monitoring changes in blood haemoglobin (Hb) and hematocrit (Hct), which are caused by a specific intervention. A mathematical equation is then used to assess the percent change in plasma volume; the equation of Harrison (1985) is one of the most often used (Equation 2) and was also used in the present study.

$$\Delta PV\,(\%) = \left(\left(\frac{Hb_1 * (1 - Hct_2)}{Hb_2 * (1 - Hct_1)} \right) - 1 \right) * 100 \qquad \text{Equation 2}$$

Where: Hb_1 = haemoglobin (g/L) measured before the intervention;
Hb_2 = haemoglobin (g/L) measured after the intervention;
Hct_1 = hematocrit measured before the intervention;
Hct_2 = hematocrit measured after the intervention.

2.5.3 Assessment of urine specific gravity

Dehydration reduces the amount of water, which is filtered through the kidneys into the bladder, therefore under conditions of progressive dehydration, urine demonstrates acute changes in its specific gravity. Under normal conditions, urine is characterized by a specific gravity (Usg) of 1.020 and has a pale to light yellow colour (Strasinger, 1994).

Urine specific gravity (Usg) can be determined with different methods and one of them is the use of reagent strips. A reagent strips consist of a chemically-impregnated absorbent pad, which is attached to a plastic strip. When the absorbent pad comes in contact with urine, a colour-producing chemical reaction takes place and the colour of the reagent pad changes in relation to the specific gravity of urine.

The absorbent pads contain different polyelectrolytes, such as bromthymol blue, poly methyl-vinyl-ther/maleic anhydride and sodium hydroxide, which have been used to determine Usg in the present study. Polyelectrolytes act as indicators of the specific gravity of urine, as they change colour differently, if they are placed in basic or acid solutions. With dehydration, Usg is altered because the ionic composition of body fluids (urine) increases, which alters pKa, i.e. the negative decimal logarithm of the acid dissociation constant of urine. Consequently, in contact with urine the colour of the reagent strips containing polyelectrolytes in the absorbent pad changes accordingly.

In the presence of suitable polyelectrolytes, the colour of absorbent pad usually changes from deep blue-green in urine of low ionic concentration, through green and yellow-green in urine of increasing ionic concentration, i.e. with increasing specific gravity of urine. The colour of the reagent strip is then compared to a standard colour chart to allow the determination of Usg. Usually, this method allows Usg readings between 1.000 (alkaline), and 1.030 (acid) in steps of 0.005.

2.5.4 Bioelectrical impedance measurements

Bioelectrical impedance is a recently developed non-invasive method, which is supposed to enable direct monitoring of a fluid status in humans (Piccoli et al. 1994) and has been recommended for the assessment of clinical conditions, which include alterations in hydration status (Kyle et al., 2004b). The method assesses the electrical properties of tissues in order to provide clinically relevant information. The reports suggest that the majority of bioimpedance analyzers have a precision of approximately 1.5 % when testing humans (Baumgartner, 1996). Several different methods of bioelectrical impedance have been described; the two of them, presented below, were used in the present study.

2.5.4.1 Classical bioelectrical impedance analysis (BIA)

BIA is based on measurements of electrical impedance to an alternating (50 kHz), low intensity (800 μA) electrical current. Electrical impedance is a combination of resistance and reactance, with resistance (R; ohm, Ω) being an opposition force to the electrical current through intra- and extracellular ionic solutions, and reactance (Xc; ohm, Ω) being the dielectric or capacitive component of cell membranes, organelles and tissue interfaces (Picolli, 2005). The reactance (Xc) is termed as inductive if $Xc > 0$, capacitive if $Xc < 0$, and if $Xc = 0$, the electrical circuit is purely resistive (Piccoli, 2005). Electrical impedance is thus affected both, by water and electrolyte content of the body.

Bioelectrical impedance analysis is supposed to assess body composition based on the fact that a body with a higher hydration and a smaller amount of fat tissue would provide smaller resistance to the electrical current than dehydrated tissue or tissue with a higher amount of fat. Namely, lean tissues contain large amounts of water and conducting electrolytes, are as such highly conductive and therefore represent a low resistance for electrical current. In contrast, fat and bone tissue contain low amounts of fluid and conducting electrolytes, are therefore poorly conductive and thus have a higher resistance for electrical current. Dehydration of the body should thus result in a higher resistance to electrical current.

2.5.4.2 BIA vector analysis

The method of BIA vector analysis is based on the plot of the two impedance vector components, thus resistance (R) and reactance (Xc), both normalised to height, in a RXc graph (Piccoli, 1994). The graph consists of point/points representing the R and Xc values, and also depicts the reference values for these two parameters; these are the elliptical probability regions with confidence intervals of 75% and 95% for a healthy population (Figure 9).

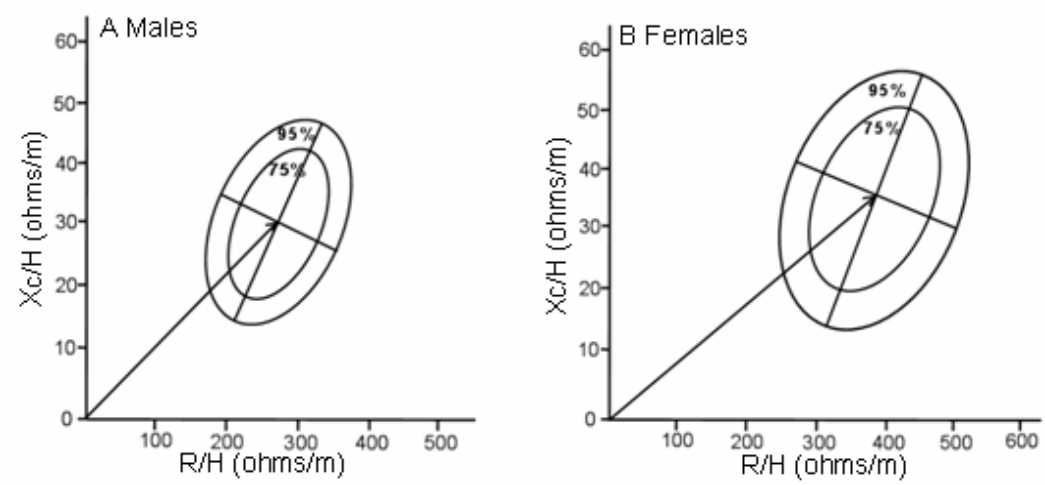

Figure 9: *BIA vectors plotted in RXc graphs for average healthy subjects, with the reference values for healthy population presented by 95 % and 75 % tolerance ellipses. The reference values are gender-specific, therefore a different graph is presented for (A) males and (B) females. R - resistance (Ω), Xc - reactance (Ω), H-height (m) (Modified from Piccoli et al, 1994).*

The RXc graph is supposed to depict both, soft tissue hydration and soft tissue mass (Piccoli et al. 2005). According to Piccoli et al. (2005), clinical information on hydration status is obtained through patterns of BIA vector distribution; BIA vector displacements perpendicular to the major axis of the ellipse was proposed to indicate changes in soft tissue mass, and BIA vector displacements parallel to the major axis of the ellipse progressive changes in soft tissue hydration (Figure 10).

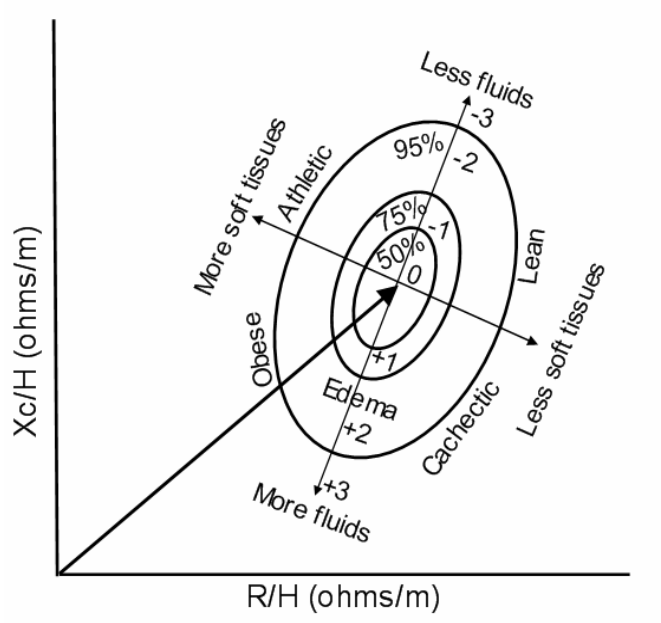

Figure 10: *The presentation of the proposed clinically relevant information obtained from BIA vector displacement in a RXc graph, The RXc is presented with elliptical probability regions for healthy population (50 %, 75 % and 95 % tolerance ellipses). R - resistance (Ω), Xc - reactance (Ω), H- height (m). (Modified from Piccoli, 2005).*

The present thesis will try to assess, which of the described methods is sensitive enough to detect mild changes in hydration status. It is expected that the results of the study will provide some useful information about the optimal method for the assessment of hydration status, which could be used as a countermeasure against dehydration and dehydration-related health risks in a general population.

2.6 Aim of the study

The aim of the study is to compare the sensitivity and practicability of five different methods for the assessment of dehydration. These methods will be used to examine how moderate environmental temperature conditions affect the maintenance of hydration status in physically active humans. Secondarily, the study will examine whether it is possible to maintain an optimal hydration status by reacting solely to the stimulus of thirst during the physical activity.

2.7 Hypotheses

Null Hypotheses:

H0a: All methods used for dehydration assessment in the present study will detect dehydration.
H0b: Hydration status can be adequately maintained during moderate physical activity in moderate-temperature environment by voluntary fluid consumption.

Alternative Hypotheses:

H1a: Not all methods used for dehydration assessment in the present study will detect dehydration.
H1b: Hydration status can not be adequately maintained during moderate physical activity in moderate-temperature environment by voluntary fluid consumption.

3. METHODS

3.1 Ethical clearance

The protocol of the study was approved by the National Ethics Committee of the Republic of Slovenia.

3.2 Subjects

Sixteen young, healthy volunteers, 8 males and 8 females participated in the study. After familiarization with the equipment and study protocol, the subjects provided a written consent for participating in the study.

3.3 Protocol and instrumentation

The experiment was performed in the early summer months, on two separate days; each time eight subjects participated. The subjects reported to the laboratory in the morning. They were allowed to have breakfast on the day of the experiment, but they had to restrict from drinking coffee or tea. Upon their arrival, they were first instructed to empty the bladder and then basic anthropometrical measurements of height, weight and skinfolds were performed. The subjects then rested supine in a 24°C room for one hour, performed a 12.5 km walk outdoors, and finally rested supine for one hour. Such protocol was used because people, who are exposed to a warm environment, as for example during heat waves, usually do not only rest, but are also, at least to some extent, physically active. During the walk, eight subjects drank water *ad libitum* (normohydrated group; NH) and the other eight were not allowed to drink (dehydrated group; DH). A schematic representation of the experimental protocol is presented in Figure 11.

Five different methods of dehydration assessment were compared in the study. Before and after the walk the subjects were weighted, urine specific gravity was determined, relative plasma volume changes calculated from haemoglobin (Hb; g/L) and hematocrit (Hct) values (Harrison, 1985) were assessed, and classical bioelectrical impedance and BIA vector analysis was performed.

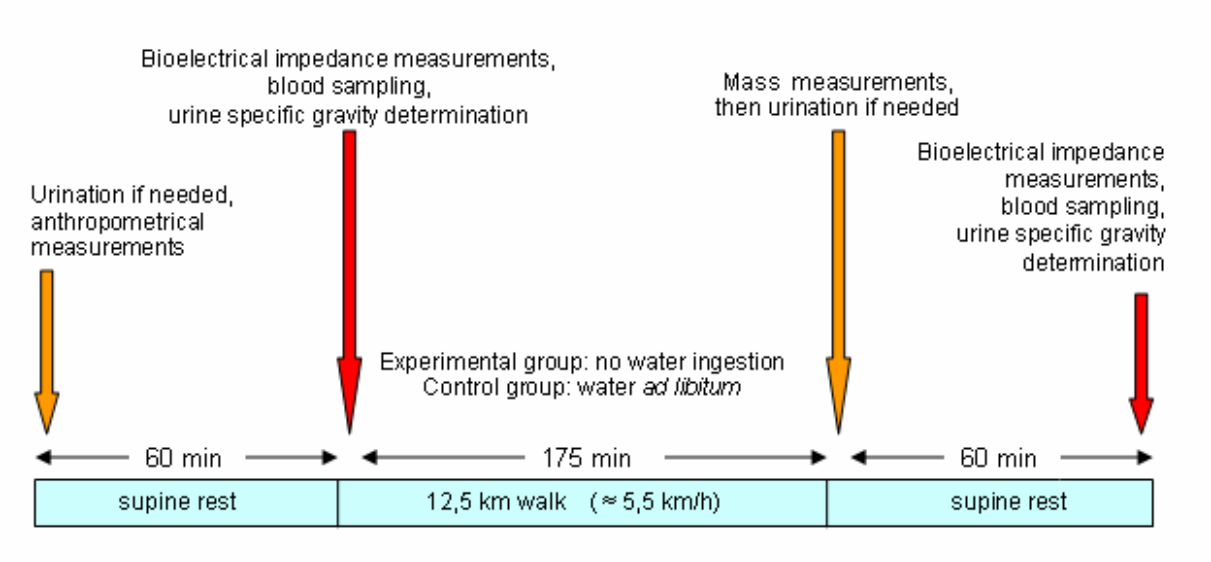

Figure 11: *A scheme of the experiment.*

3.3.1 Anthropometrical measurements

Height and body mass of the subjects were measured with an electronic scale (Vita Libela Elsi, Celje, Slovenia) with subjects wearing their underwear. According to the manufactures specifications, the precision of the electronic scale was 0.1 kg. During weighing, the subjects were required to stand still. Following the walk, the subjects towelled-off the excess sweat if needed and then the weighing was repeated.

Skinfolds were measured with a Harpenden skinfold calliper (Body Care, United Kingdom) at several body sites (chest, abdomen and thigh for males; iliac crest, triceps and thigh for females).The skinfold data were used to determine the percentage of adipose tissue in the body and lean body mass according to the prediction formula of Jackson & Pollock (1985).

Fluid loss was determined by subtracting the subjects' mass measured following the walk from the mass measured before the walk. It was adopted that a mass loss of 100 g corresponded to 100 ml of fluid loss (Cheuvront & Sawka, 2005).

Following the anthropometrical measurements, the subjects were equipped with a heart rate monitor (S625-X, Polar Electro Oy, Finland) and asked to lie down on a foam pad for one hour. It has been demonstrated that this time is sufficient to achieve

equilibrium between intravascular and extra vascular fluid compartments, and to minimize the possibility of fluid shifts and Hb redistribution during blood sampling (Nose et al., 1988). Towards the end of a one-hour rest, heart rate (HR; bt/min) was measured with a heart rate monitor (S625-X, Polar Electro Oy, Finland), systolic (SAP; mm Hg) and diastolic (DAP; mm Hg) blood pressure with a mercury sphygmomanometer (Miniature 300B, Speidel + Keller, Germany), and tympanic temperature (Tty; °C) with an infrared tympanic thermometer (TermoScan IRT 3020, Braun, Kronberg, Germany).

3.3.2 Blood sampling and determinations of plasma volume changes

Following the bioelectrical impedance measurements, a sample of venous blood was taken from the subjects by certified laboratory personnel (Figure 12). The concentration of haemoglobin (Hb), hematocrit (Hct) and plasma sodium concentration was determined in a certified laboratory (Adrialab, Ljubljana) according to the standard procedures; a photometry at 550 nm was used to determine blood Hb (Pentra 120; Horiba group, ABX, France), Hct was then calculated (Pentra 120; Horiba group), and an ion selective method, i.e. an indirect potentiometry (Roche/Hitachi 912 Autoanalyzer, Roche. Diagnostics GmbH, Germany) was used to determine the concentration of plasma sodium (Na^+; mmol/L).

The values of Hb and Hct were used to assess percent changes in plasma volume due to fluid loss according to the equation of Harrison (1985), which was presented in the chapter 2.5.2 of this thesis.

Figure 12: *Sampling of venous blood*

3.3.3 Urine specific gravity determination

The subjects provided a urine sample after the blood sampling. Urine specific gravity (Usg) was determined with reagent strips (Multistix 10 SG, Bayer Diagnostics Mfg., Bridgend, United Kingdom), which were placed in a plastic cup with fresh, uncentrifuged urine. The strips were immediately withdrawn from the urine sample, excess urine was removed, and the strips were placed on a cup for a standardized amount of time (45 seconds) so that the chemical reaction occurred. To determine the specific gravity of the urine sample, the colour of reagent strips was read on a good light source between 60 and 120 seconds following the removal of the reagent strips from the urine sample, and was compared with a standard colour scale (Figure 13).

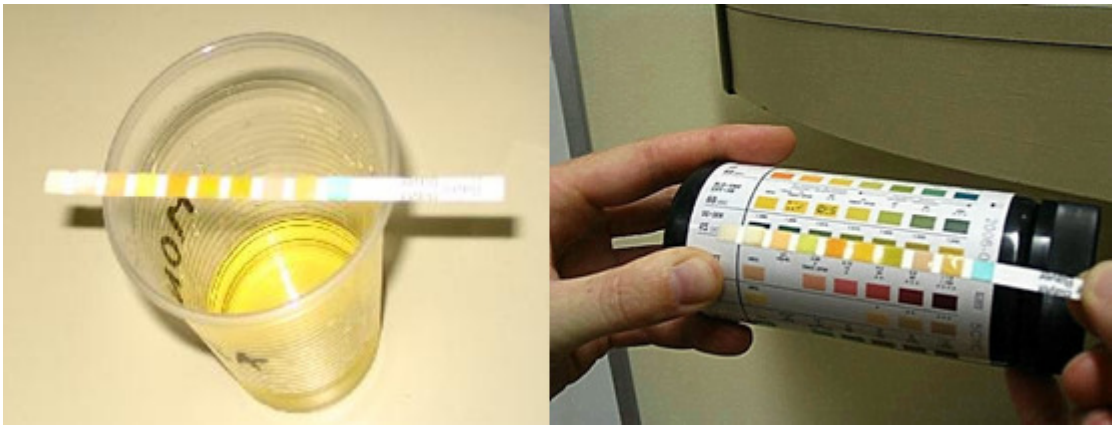

Figure 13: *Urine specific gravity determination.*

3.3.4 Bioelectrical impedance measurements

Bioelectrical impedance measurements, both classic and BIA vector, were performed immediately after a one-hour rest, both before and after the walk, with a soft tissue bioimpedance analyzer (STA, AKERN. S.r.l., Pontassieve, Italia). While the subjects were lying supine, their arms and legs were positioned at an angle of 45^{o} to the body axis, to avoid the contact of upper and lower extremities with the trunk, which might interfere with the bioimpedance measurement (Figure 14). After skin cleaning with alcohol wipes, two electrodes were attached to a non-palmar side of the hand, and two to the non-plantar side of the foot. A low-intensity alternating electrical current (800 μA, 50kHz) was then circulated through the electrodes and the induced resistance (R, Ω) and reactance (Xc, Ω) were measured.

Figure 14: *Bioelectrical impedance measurements.*

The software for body composition analysis (Bodygram 1.21, AKERN, Pontassieve, Italia) developed by the manufacturer of the bioimpedance analyzer, assessed several different parameters, such as the percentage of total body water (TBW; L), intracellular (ICW; L), and extracellular (ECW; L) fluid content using the recorded electrical (resistance and reactance) and anthropometrical data (age, height, body mass and gender).

3.3.5 Experimental procedure

Following these initial measurements, the subjects performed a 12.5 km walk of a moderate intensity (average speed ≈ 5.5 km/h) outdoors in a moderately warm environment (Ta = 20(4)^{o}C). Heart rate of the subjects was measured in 15-minute intervals. During the walk, the subjects wore long trousers and long-sleeved shirts. The course of the walk is presented on Figure 15.

Throughout the walk, one group of subjects was restricted from drinking (dehydrated group), and the other one was allowed to drink *ad libitum* (normohydrated group). Such protocol was used to induce different hydration status of the subjects and to assess their fluid replenishment capacity. Furthermore, the water bottles were weighed before and after the walk, to calculate the amount of consumed water.
Air temperature, relative humidity and barometric pressure data for the location of the walk were obtained from the Environmental agency of the Republic of Slovenia.

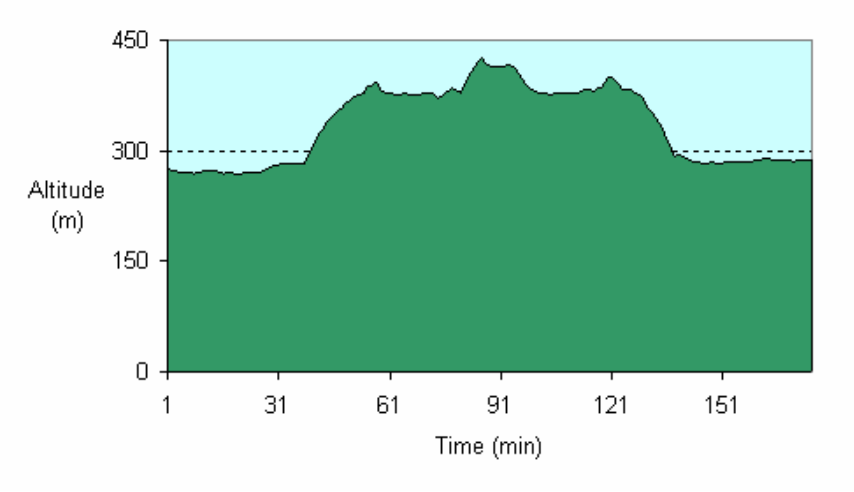

Figure 15: *The course of the walk.*

Following the walk, the same measurements were performed over a similar time-scale as prior to the walk.

3.4 Statistical analysis

We expected a change of 0 % of body mass in the control group and a change of up to 3 % of body mass in the experimental group. With a common standard deviation of 1 % for both groups, and the significance level of 0.05, the number of subjects, required to achieve the power of a one-sided statistical test of 0.9 was 2.93 (UCLA Department of Statistics, 2004). The number of subjects, which participated in the study, was thus sufficient to reach the stated requirements.

Body mass, SAP, DAP, tympanic temperature, and the bioimpedance parameters (TBW, ICW, ECW, Rz, Xc), Hb, Hct, plasma sodium and Usg were analysed with a multifactorial ANOVA with repeated measures on one factor. A Student T-test for independent samples was used to determine differences between normohydrated and dehydrated group for the anthropometrical measurements (age, height and weight), body fat tissue mass, changes in body mass during the experiment, and plasma volume change. P values of less than 0.05 were adopted as statistically significant. All data are presented as mean (SD).

4. RESULTS

4.1 Anthropometrical measurements

Subjects' mean (SD) age was 24 (4) years, height 176 (8) cm, mass 69.5 (11.1) kg, and percentage of body fat 18.4 (7.3) %. The normohydrated and dehydrated group did not differ significantly in any of these parameters (P>0.05).

The subjects in the normohydrated group ingested 1094 (208) g of water during the walk. Pre- and post walk body mass of both normohydrated and dehydrated subjects, as well as the absolute mass loss, and percent change in body mass in both groups is presented in Table 2. Mass loss differed significantly (P<0.001) between the two groups, with a greater mass loss observed in the dehydrated group. Consequently, the percent change in body mass reduction was also significantly higher (P<0.01) in the dehydrated, as compared to the normohydrated group.

Table 2: *Mean (SD) mass of the normohydrated and dehydrated subjects before and after the walk, and the mass loss induced by the experimental protocol. **P<0.01; *** P<0.001*

	Mass before (kg)	Water consumption during the walk (g)	Mass after (kg)	Mass loss (kg)	Percent change in body mass (%)
Normohydrated group	70.8 (12.9)	1094 (208)	70.2 (12.6)	0.6 (0.8)	0.8 (1.0)
Dehydrated group	68.6 (14.0)	0 (0)	67.0 (13.8)	1.6*** (1.0)	2.3** (1.5)

4.2 Plasma volume changes

Haemoglobin (Hb; g/L) decreased significantly (P<0.002) in both groups after the walk, as compared to the pre-walk values, however, no statistically significant differences (P>0.05) in Hb were observed between the normohydrated and dehydrated group, nor before or after the walk (Table 3).

Similarly, hematocrit (Hct) was significantly (P<0.03) lower after the walk in both groups (Table 3), but the absolute changes were small and the difference between normohydrated and dehydrated group was not statistically significant (P>0.05).

In contrast to the expectations, plasma volume, as calculated from Hb and Hct values, increased in both groups after the walk. The calculated percentage of plasma volume change, however, did not differ significantly between the normohydrated and dehydrated group (P>0.05).

Table 3: *Blood parameters measured before and after the walk. Mean (SD) values for haemoglobin (Hb; g/L), hematocrit (Hct), calculated plasma volume change (PV change; %) and plasma sodium (Na^+; mmol/L) are presented for 8 subjects in both experimental groups; plasma sodium is presented for 7 subject as the measurements of plasma sodium were incorrect for one of the subjects in each experimental group.*

	Hb (g/L)		Hct		Calculated PV change (%)	Na^+ (mmol/L)	
	before	after	before	after		before	after
Normohydrated group	134 (13)	130 (14)	0.40 (0.04)	0.39 (0.03)	5.73 (6.67)	140 (1)	139 (1)
Dehydrated group	133 (6)	131 (7)	0.40 (0.02)	0.39 (0.02)	3.36 (3.67)	139 (2)	142 (3)

4.3 Plasma sodium

Mean plasma sodium (Na^+; mmol/L) did not change significantly in any of the two groups after the walk, as compared to the pre-walk values (P>0.05). Furthermore, the plasma sodium did not differ significantly (P>0.05) between the dehydrated and normohydrated group (Table 3), nor before or after the walk.

4.4 Urine specific gravity

Following the walk, urine specific gravity (Usg) increased significantly in both groups (P<0.001), as compared to the values obtained prior to the walk. Furthermore, Usg was significantly higher (P<0.03) following the walk in the dehydrated, as compared to the normohydrated group (Table 4).

Table 4: *Urine specific gravity (Usg) before and after the walk in the normohydrated and dehydrated group. * P<0.05*

	Usg before	Usg after
Normohydrated group	1.011 (0.09)	1.018 (0.09)
Dehydrated group	1.015 (0.07)	1.028* (0.05)

4.5 Blood pressure, tympanic temperature and heart rate

Systolic blood pressure (SAP; mm Hg) before the walk was 118 (7) mm Hg in the dehydrated, and 118 (8) mm Hg in the normohydrated group. Following the walk, SAP was 106 (19) mm Hg and 112 (8) mm Hg in the dehydrated and normohydrated group, respectively. SAP significantly ($P<0.01$) decreased after the walk in both experimental groups, but did not differ significantly between the two groups ($P>0.05$) neither before nor after the walk.

Diastolic blood pressure (DAP, mm Hg), as well as tympanic temperature (Tty, °C) of the normohydrated and dehydrated group measured at the end of a one-hour rest, both before and after the walk, were similar ($P>0.05$). Also, heart rate (HR, bpm) in both, the normohydrated and dehydrated group, did not change significantly ($P>0.05$) after the walk as compared to the pre-walk values. Mean (SD) values of these parameters are presented in Table 5.

During the walk, HR of the subjects was similar in both groups ($P>0.05$), with the average of 112 (29) bpm in the dehydrated, and 115 (26) bpm in the normohydrated group.

Table 5: *Mean (SD) values of DAP, Tty and HR of the normohydrated and dehydrated subjects, both before and after the walk.*

	DAP before (mm Hg)	DAP after (mm Hg)	Tty before (℃)	Tty after (℃)	HR before (bpm)	HR after (bpm)
Normohydrated group	72 (5)	69 (5)	36.8 (0.3)	36.7 (0.2)	63 (12)	71 (15)
Dehydrated group	77 (9)	71 (10)	37.0 (0.4)	36.9 (0.3)	57 (14)	64 (22)

4.6 Bioelectrical impedance measurements

4.6.1 Classical bioelectrical impedance (BIA)

In contrast to body mass measurements, no significant changes ($P>0.05$) were observed in the calculated parameters of total body water (TBW; L), intracellular

water (ICW; L) and body cell mass (BCM; kg), in any of the two experimental groups, as assessed by classical bioelectrical impedance method (Table 6).

Table 6: *Mean (SD) values of total body water (TBW), intracellular water (ICW), and body cell mass (BCM), i.e. the parameters provided by the automatic bioelectrical impedance analysis in the normohydrated and dehydrated subjects, both before and after the walk.*

	TBW before (L)	TBW after (L)	ICW before (L)	ICW after (L)	BCM before (kg)	BCM after (kg)
Normohydrated group	37.8 (7.6)	37.7 (6.9)	22.6 (5.0)	22.1 (4.6)	24.8 (5.5)	24.2 (5.0)
Dehydrated group	38.1 (7.3)	37.9 (7.4)	23.3 (5.0)	23.0 (5.1)	25.5 (5.5)	25.3 (5.6)

Extracellular water (ECW; L), another parameter assessed by automatic bioelectrical impedance analysis, was significantly higher ($P<0.001$) after the walk in both groups. It increased from 15.2 (2.8) L to 15.6 (2.6) L in the normohydrated, and from 14.8 (2.4) to 14.9 (2.4) in the dehydrated group. The observed change in ECW was significantly higher ($P<0.05$) in the normohydrated, as compared to the dehydrated group.

4.6.2 BIA vector

The two components of the BIA vector analysis, thus resistance (R; Ω/m) and reactance (Xc; Ω/m), were analysed independently in both groups.

The resistance component of the total bioelectrical impedance (normalized to height of the subjects) (R/h; Ω/m) decreased from 363 (79) Ω/m before the walk to 352 (66) Ω/m after the walk in the normohydrated group, and from 345 (53) Ω/m to 340 (62) Ω/m in dehydrated group; this difference, however, did not reach the level of statistical significance in any of the two groups ($P>0.05$). Furthermore, no statistically significant differences ($P>0.05$) in the resistance component were observed between the two groups, nor before or after the walk.

Reactance (Xc/h; Ω/m) decreased from 40 (7) Ω/m as measured before the walk to 38 (6) Ω/m after the walk in the normohydrated group, and from 40 (6) Ω/m to 39 (6) Ω/m in dehydrated group; this difference was statistically significant ($P<0.001$) in

both groups. However, no statistically significant differences (P>0.05) in Xc/h were observed between the two groups, nor before or after the walk.

The observed values of resistance (R/h;Ω/m) and reactance (Xc/h; Ω/m) were used in a RXc graph and are presented in Figure 16.

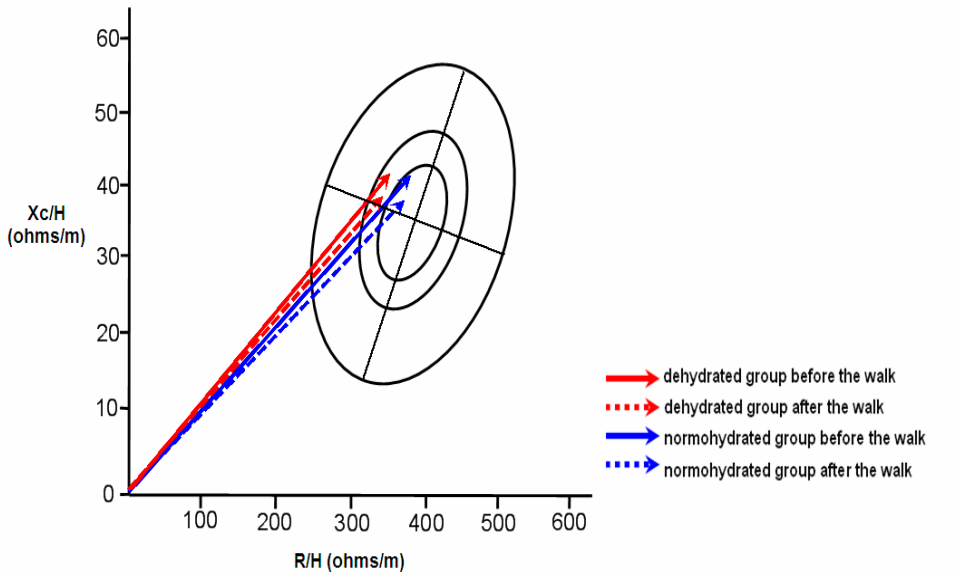

Figure 16: *BIA-vector graph presenting data from the dehydrated (red lines) and normohydrated (blue lines) group, both before (full lines) and after (dotted lines) the walk. R - resistance, Xc - reactance and H - height.*

5. DISCUSSION

It has now been demonstrated with high certainty that human environment is changing rapidly, and that significant changes in climate have been observed in the last decades. One of the most serious challenges the humanity is facing today, is global warming, which is caused by human activities, and because of which the ambient temperature has increased significantly in the last century (Houghton et al., 2001). Undoubtedly, an increase in the ambient temperature affects human performance, well-being and health; furthermore, with the continuation of global warming in the future, the humanity will be more often exposed to extreme weather conditions, with all the adjoining heat-related health risks.

Some direct evidence that global warming is already affecting human well-being and health has already been presented (Alberdi et al., 1998; Arnfield, 2003; Davis et al., 2003). It has been demonstrated that an increase in ambient temperature can cause different heat-related health incidents and can result in higher mortality during heat waves (Donaldson et al., 2001). According to the report of the European Environmental Agency (EEA, 2005), 35000 heat-induced deaths were registered in Europe during the 2003 heat wave; the majority of them have been attributed to both, cardiovascular problems and dehydration. Thus, dehydration has been recognized as one of the most common heat-related problems, and a prominent cause for the reported increases in mortality during heat waves (EEA, 2005). It therefore seems necessary to evaluate, what countermeasures one can provide to avoid it.

During everyday life, extreme levels of dehydration are usually not experienced, but dehydration can present a serious health risk, particularly with prolonged exposure to a stressful environment and/or with in the high-risk populations if it is not detected at its early stage. Rather surprisingly, although the problem of dehydration is not a new one, no specific universal method for the detection of dehydration has yet been established. The present study therefore compared five different existing methods for the assessment of mild dehydration and tried to identify a method which is sensitive enough to detect small changes in hydration level. Furthermore, the present study assessed the risk of dehydration during daily activities, as well as the capacity for the

replenishment of water losses by drinking *ad libitum*, as this risk will most likely increase in extreme weather conditions. The discussion will therefore focus first on the comparison of different methods for the assessment of dehydration, and second, on the water replenishment capabilities of the subjects involved in the present study.

5.1 Comparison of different methods for the assessment of dehydration

Mass loss

Most often it is assumed that the mass loss observed after exercise corresponds to the water loss; the latter results from sweating, insensible perspiration and also from respiratory water loss - among these processes, sweating is the most important cause for dehydration. In the present experiment, body mass decreased after the walk with all 16 subjects, thus it can be adopted that body mass measurements detected water loss both dehydrated and normohydrated group.

There is one confounding factor, however, which can affect the estimation of dehydration through mass measurements, particularly when dehydration is associated with exercise. Namely, during exercise body mass can decrease not only because water is being lost, but also because the amount of glycogen stores and fat in the body decrease. Namely, glycogen is a molecule that stores both energy and water. It is a source of energy which is synthesized when blood glucose concentration is high and it is stored in muscles and liver. In contrast, when blood glucose levels decrease, glycogen is metabolized into glucose and then used as a source of energy (Berg et al, 2001). Thus, during exercise, glycogen is being used to provide energy needed to perform muscular work. However, with the energy storage capacity of 1 kcal/g, glycogen is a very ineffective form of energy storage, therefore fat, which has a storage capacity of 9 kcal/g (Sawka et al, 2005) is also used as a source of energy during prolonged exercise; both glycogen depletion and fat oxidation contribute to mass loss during exercise.

It was reported that the highest value of fat oxidation is approximately half of gram per minute (Mrak et al, 2006). Furthermore, according to some estimations (Mrak et

al, 2006), a non-athlete person who walks at a speed of 6 km/h, can oxidise as much as 20 g of fat per hour. According to such an estimation and the average speed of walking in the present study (5,5 km/h), it is expected that the subjects oxidised approximately 18 g of fat per hour. Furthermore, in order to evaluate mass changes due to glycogen depletion during exercise, one should assess the work intensity of the subjects. In the present study, a very rough approximation of the subjects' work intensity can be obtained by comparing the subjects' heart rates measured during the walk with their arbitrary maximum heart rates calculated as (220-age). According to such very rough approximation, in the present study the subjects exercised at approximately 57% of their maximal heart rate, thus at approximately 40 % of their maximal oxygen uptake (Åstrand et al., 2003). With such work intensity, the amount of metabolized glycogen is expected to be less than 66 g/per hour (van Loon et al., 2001).

Considering all the above, water loss, thus dehydration, was the largest contributor to body mass loss in the present study. Nevertheless, as the rate of glycogen depletion increases with increasing exercise intensity, the effects of glycogen and fat oxidation upon body mass have to be considered whenever measurements of body mass are used to assess dehydration.

According to the results of the present study, monitoring of body mass using a standardized protocol proved to be a practical, non-invasive, sensitive and valid method for hydration testing. It has to be noted, however, that body mass measurements must be performed on a certified scale, with subject wearing minimal clothing, no shoes and having an empty bladder, and when the excess sweat has been towelled-off. The accuracy of pre- to post-exercise change in body mass for the estimation of total fluid loss due to sweating can be further increased by monitoring the total fluid intake and fluid excretion and provided that glycogen and fat oxidation are not a serious confounding factor. If all of the above is considered, it can be concluded that mass monitoring is an effective tool for detecting dehydration even at low dehydration levels. The limitation of the method is, however, that none of the mass values can be representative of a "normohydrated" mass without previous measurements or additional information.

Plasma volume

Relative changes in plasma volume were also used in the present study to assess the dehydration level of the subjects. It is generally accepted that when a person is dehydrated, plasma volume decreases (Dill and Costill, 1974; Harrison, 1985), and studies have demonstrated that the magnitude of the reduction in plasma volume is roughly proportional to the simultaneously observed decrease in body mass (Saltin, 1964, Costil and Fink, 1974; Nunneley, 1979).

It has been suggested that if heat and exercise are used to induce dehydration, plasma volume must be measured after the thermal stress has been removed, after the body temperature has returned to normal, and without replacement of fluid losses (Harison, 1985). In the present study, all the subjects rested supine for one hour in a thermoneutral environment after the walk. As the tympanic temperature after an one-hour rest did not differ between pre- and post walk conditions, and none of the subjects ingested any fluids during the rest, all of the upper mentioned requirements for the assessment of plasma volume changes were fulfilled.

It has been demonstrated that during dehydration, the amount of water lost from plasma is five times higher than the amount of water lost from other fluid compartments (Senay, 1979). However, in contrast to expectations, no relative decrease in plasma volume was observed in the present study in any of the two experimental groups. Even more, with respect to the pre-exercise values, thus at approximately 60 minutes after supine rest following the walk, a relative increase in plasma volume was observed in both experimental groups. This result, although unexpected, can be explained considering the factors that affect plasma volume. Namely, it has been demonstrated that after heat exposure (Beaumont et al., 1974, as cited by Harrison, 1985), or exercise (Harrison et al, 1978) which result in dehydration, and a subsequent return of the subjects to a cooler environment or rest, plasma volume tends to be restored even if dehydration is maintained (Beaumont et al, 1974). This restoration of plasma volume is most likely achieved by redistribution of water within body fluid compartments, so that the effect of water deficit is minimized (Sawka and Montain, 2000).

Furthermore, there exists an additional important condition which affects the assessment of plasma volume changes, i.e. body posture. Namely, body posture affects the fluid shifts between different fluid compartments and consequently plasma volume; if a subject is in an upright position, haemoconcentration will appear, and if positioned supine, haemodilution will follow. Consequently, any observed change in plasma volume may be related to body posture, exercise-, and/or heat-induced dehydration, or the combination of various factors (Hagan et al, 1978).

In the present study, the effect of posture was considered with blood sampling; both, before and after the walk. Blood sampling was performed in identical, stable and supine conditions, so that body posture could not have affected the measurements. As it was demonstrated that posture-induced changes in plasma volume occur rapidly and are virtually complete within 20 minutes (Hagan et al, 1978), the fluid redistribution must have been completed at the end of an one-hour rest in the present study. Nevertheless, the observed relative changes in plasma volume did not reflect the actual changes in hydration level, as detected by mass loss and urine specific gravity. It is expected that fluid loss induced by exercise, resulted in an increased plasma osmolality after the walk, because of which water shifted from extracellular to intravascular fluid compartment, and possibly even from intracellular to extracellular fluid compartment. It seems reasonable to assume that these inter-compartmental fluid shifts masked the reductions in plasma volume induced by exercise in the present study.

The results of the present study demonstrate that relative changes in plasma volume as assessed from haemoglobin and hematocrit values can not be used for the assessment of dehydration, if they are not performed immediately after exercise. The results are in compliance with the report of Harrison (1985), who suggested that the measurements of plasma volume, which are performed with a certain lag period after the end of exercise, can not represent the actual changes in plasma volume that occur during or immediately after exercise. Thus, even if the effects of body posture on plasma volume are taken into the account, the measurements of plasma volume may not be suitable for the assessment of dehydration, if the blood sampling is not performed immediately after the end of exercise. In this case, however, the condition

of thermal neutrality required for the assessment of plasma volume (Harrison, 1985) can not be fulfilled.

Last but not least, the measurement of plasma volume changes by blood sampling is an invasive, rather expensive and time consuming technique, which requires trained laboratory personnel for blood sampling. The invasive procedure also causes a small but significant risk of infection, bruising and vein damage.

Urine specific gravity

Determining urinary indices, including urine specific gravity (Usg) is one of the most widely used methods for the detection of dehydration (Adam; 1997; Kavouras, 2002). Namely, the major contents of urine are urea, chloride, sodium, potassium, phosphate, uric acid, sulphate and a variable amount of water (Pradella, 1988), the amount of which depends on the hydration level of the subject. Through regulating electrolytes and water excretion by the kidneys, the human body can maintain the homeostasis of osmotic pressure, thus, with dehydration less water is excreted by the kidneys, therefore urine is concentrated, its osmolality and Usg increase and consequently its colour becomes darker (Lennert and Luft, 1997).

A few guidelines that relate urine specific gravity with the hydration level have already been proposed; National Collegiate Athletic Association (NCAA), for example, suggested that urine specific-gravity of <1.020 indicates euhydration (NCAA, 1998). Similarly, Popowski et al. (2001) reported that Usg values of 1.006 to 1.020 represent an euhydrated state. Thus, a subject is considered normohydrated, if urine specific gravity is <1.020, and dehydrated if urine specific gravity exceeds 1.020 (Stuempfle and Drury, 2003). Nevertheless, it has not yet been clearly identified, what is the level of dehydration that can be successfully detected by Usg measurements; the results of the present study demonstrate that the method of urine specific gravity determination can be successfully used to detect even low levels of dehydration. Namely, Usg measurements increased significantly in the dehydrated group after the walk, and the corresponding Usg exceeded the value of 1.020, which has been classified as a point of dehydration (NCAA, 1998). In contrast, although Usg also increased after the walk in the normohydrated group in the present

experiment, the Usg remained below 1.020. Thus, according to the proposed NCAA boundary for dehydration (NCAA, 1998), the normohydrated group, who ingested water *ad libitum*, did not suffer dehydration, despite the fact that the subjects did not ingest enough water to replace all the fluid lost during exercise.

According to the results of the present study, urine specific gravity determination proved to be a relatively inexpensive, simple, and quick measurement, which can be easily used by inexperienced people. Furthermore, the results of the study prove that the used method of Usg determination can be successfully used for the detection of even small changes in hydration status. Although the readings of colour of the absorbent pad on the reagent stick are somewhat subjective, which can be considered as a weakness of this method, the use of refractometers, devices for the assessment of Usg, which determine Usg independently of a colour reaction, can further reduce such potential methodological errors and improve the susceptibility of the method.

Classical bioimpedance measurements (BIA)

During the past decade, evidence has been gathered that supported the use of bioelectrical impedance analysis (BIA) for monitoring of the hydration status (Piccoli et al, 1994). BIA was described as an indirect method to measure body fluid volumes and body fat content of various groups of people. Furthermore, it was reported as a safe, convenient, noninvasive, rapid and portable method of estimating body composition (van Loan, 1990; Kushner, 1992).

However, some studies stressed that many factors, such as electrode placement, side of the body, limb position, body posture, ambient and skin temperature, plasma osmolality and sodium concentration could at least potentially alter BIA measurements (Koulmann et al, 2000). Furthermore, when the extent of body water compartments is subject to acute changes, BIA was reported not to allow accurate assessments of TBW and ECW (Battistini et al, 1993; De Lorenzo et al, 1994). It was suggested that one of the main limitations of BIA seems to be that its accuracy is highly dependent on the selection of appropriate prediction equations; these should be as specific as possible to the monitored study group in terms of age, sex, ethnicity, body fat content and health status (Baumgartner, 1996). Nevertheless, several studies

(Piccoli et al, 1994; Yannakoulia et al, 2000; Kyle, 2004b) concluded that if the stated factors are considered and the required conditions fulfilled, BIA can be used for accurate estimation of the total body water composition.

Therefore, in the present study BIA was used to evaluate the distribution of water among body fluid compartments, both before and after the changes in body hydration induced by the experimental protocol, and the recommendations mentioned in literature were considered. Nevertheless, BIA did not detect dehydration in any of the experimental groups, as evident from similar total body water, intracellular water and body cell mass parameters obtained before and after the walk. Even more, the parameter of extracellular water, as determined by BIA, was significantly higher after the walk in both groups, which might suggest the existence of fluid shifts between different fluid compartments.

The results of the present study are in agreement with the reports of Kyle et al. (2004a,b) and O'Brien et al. (2002), who reported that BIA is not valid under conditions of significantly altered hydration, because the equations used in BIA were not developed for individuals with altered hydration status. Furthermore, changes in electrolyte balance associated with dehydration seem to influence BIA measurements (Kyle et al, 2004a), because any change in electrolyte content also affects the ratio of intra- to extracellular water shifts, which in turn influences the resistance component of BIA assessment (Kyle et al, 2004a).

The results of the present study thus contradict to the use of BIA for the assessment of hydration status. It seems that bioelectrical impedance analysis has not been sufficiently standardized and that it does not include a sufficient quality control procedure. Therefore, the results of the present study suggest that classical bioimpedance measurements cannot be used for monitoring changes in hydration status.

BIA vector

In contrast to classical bioimpedance parameters, direct measurements of impedance do not depend on prediction equations or models. To assess bioelectrical impedance,

the values of resistance (R) and reactance (Xc), usually standardized for height, are first measured and then plotted in the R/Xc plane as a BIA vector. An individual vector is then compared to tolerance elliptical values calculated for the healthy population of the same gender and race, and several studies (Piccoli et al, 1997; Utter et al, 1999; Jebb et al, 2000) demonstrated that BIA vectors falling outside the 75% tolerance ellipse indicate abnormal tissue impedance, which can result from changes in hydration status. In case of dehydration, which decreases the conductibility for the electrical current through the body, a shift to higher resistance and higher reactance, indicating less fluids and a reduction in soft tissue mass, respectively, is expected (Piccoli, 2005).

The results of the present study demonstrate that the BIA vector method does not reliably detect changes in hydration status. Namely, when comparing the values of resistance and reactance before and after the walk, the reactance decreased, which, according to the literature, would indicate a reduction in soft tissue mass, and resistance (non-significantly) decreased, which, according to the literature, would indicate an increase in hydration, despite the undisputable fact that dehydration of the subjects was induced by the experimental procedure. Furthermore, the BIA vector method did not discriminate between the two groups in any of the impedance parameters. It is therefore concluded that, similar to the classical bioimpedance analysis, the BIA-vector method is inappropriate for monitoring of the changes in hydration status.

5.2. Water replenishment capacity

Apart from comparing the methods for the assessment of dehydration, the present study tried to assess briefly whether it is possible to replace adequately the fluid lost by exercise with *ad libitum* water ingestion. Ad *libitum* water ingestion results from the stimulus of thirst, and it has been demonstrated that to elicit thirst, fluid losses of 1% to 2% of body mass are necessary (Casa, 1999).

It is generally recognized that adequate fluid ingestion is necessary for the maintenance of plasma volume, which is important for the optimal regulation of

both, blood pressure and body temperature during exercise in a hot environment (Fortney et al, 1981; Nadel, 1984). It is therefore essential to replenish fluid loss and to maintain an adequate plasma volume in any set of circumstances for the proper function of the cardiovascular and thermoregulatory system (Sawka and Montain, 2000). Furthermore, maintaining water balance is critical for the function of all organs and for maintaining health in general (Sawka, 1988; Mack and Nadel, 1996), as water provides the medium for biochemical reactions within cells.

Both, exercise and heat stress cause fluid imbalances that need to be corrected (Marriott, 1993; Convertino et al, 1996; Montain et al, 1996). A dehydrated person who would exercise in the heat, even a person with high aerobic capabilities, will incur significant adverse effects (Sawka, 1992), because dehydration increases physiological strain, decreases exercise performance, and negates the thermoregulatory advantages conferred by high aerobic fitness (Buskirk et al, 1958, Cadarette, 1984) or heat acclimation (Sawka et al, 1983), such as an increased rate of sweating. It is generally recognized that if strenuous exercise is performed by a dehydrated person, the medical consequences can be devastating (Hales et al, 1996).

Several studies (Pitts and Consolazio, 1944; Ladell, 1955; Coyle and Montain, 1992a,b) reported that dehydration by less than 3 to 4 % of body mass, which occurs during prolonged exercise in the heat will impair the performance and may cause heat exhaustion and collapse. Others (Wyndham and Strydom, 1969), however, interpreted that dehydration of less than 3 to 4% of body mass causes only insignificant impairment of physiological performance and is as such tolerable, and that it is the dehydration of more than 4 % of body mass, which is dangerous to health.

In general, people dehydrate during exercise in the heat because they do not have any fluids available, or because their response to the stimulus of thirst is inadequate (Sawka and Pandolf, 1990; Greenleaf, 1992). In such cases, a person is euhydrated at the beginning of exercise, but incurs dehydration over a prolonged period of time. A similar situation can also be expected with prolonged exposure to warm environment, for example during heat waves. It has been reported that if a relatively mild

dehydration of 1 to 2 % of body mass occurs frequently, dehydration can become chronic (Casa, 1999). However, other reports suggest that even when daily water losses are significant, with body mass loss of up to 4 % (Costill et al, 1975), and the recovery periods of drinking *ad libitum*, which are combined with food intake and plentiful electrolyte replacement (Szlyk et al, 1990; Maughan et al, 1996) are long enough (24 hours), the restoration of normal, nomohydrated state is possible (Sawka et al, 2005).

One of the most recent position stands (Convertino et al., 1996) of the American College of Sports Medicine (ACSM) recommended that a physically active person exercising in a warm environment should initiate drinking before the actual feeling of thirst is induced in order to prevent dehydration. Other authors (Barr and Costill, 1989; Montain et al, 1996; Noakes, 2003a) have been critical to such guidelines, stating that drinking excessively large volumes of fluid during exercise can induce a hyponatremic condition, and suggested that responding to the stimulus of thirst may be a sufficient drive for the maintenance of optimal hydration. Again, others have disagreed, stating that the ingested fluid is first stored in the stomach, which may temporarily cause people to gain body mass above the euhydrated levels, however the volume of the intracellular and extracellular fluid compartments will not increase until the ingested fluid moves into the intestines for absorption and distribution throughout the body (Coyle, 2004).

It now seems that a general consensus has been forming, suggesting that fluid should be ingested at rates that most closely match the sweating rate, and that unrestricted drinking that causes people to gain body mass and body water, thus hyperhydration, should be discouraged (Coyle, 2004). Thus, fluid should not be ingested at rates in excess of sweating rate and body mass should not increase with fluid replenishment during exercise (Coyle, 2004).

In the present study, the normohydrated group, who ingested water *ad libitum,* replaced only 49 % of the body mass, which was lost during the walk. Although some of the observed mass loss can be attributed to glycogen and fat oxidation, it is expected that the majority of the observed mass loss was due to water loss.

Hargreaves et al. (1996) reported that if people drink during exercise, less muscle glycogen is used. According to several reports (Pitts and Consolazio, 1944; Hubbard et al, 1984; Broad, 1996; Sawka et al, 2005), a person's physiological drive for fluid intake during exercise compel people to drink at a rate that replaces approximately 30 to 70 % of their fluid losses, which is in agreement with the results of the present study. The concept that thirst does not drive people to ingest fluid at a rate of fluid loss during exercise has been called voluntary dehydration (Coyle, 2004).

Nevertheless, if one considers the observed mass loss in the normohydrated group, who ingested water ad libitum, the mass loss was on average less than 1 %; it thus remained below the level of 2 %, which according to Sawka and Montain (2000), presents a normal range of hydration during exercise.

Furthermore, it has to be noted that the fluid available for drinking to the normohydrated group in the present study was pure water of ambient temperature. Namely, beside thirst, the important factors that govern fluid intake are also availability of fluids, opportunity to drink and gastrointestinal comfort (Coyle, 2004). It is expected that if the palatability of fluid would be increased by improving its flavour (Hubbard et al, 1984; Engell and Hirsch, 1999) or decreasing its temperature to the optimal 15 to 21°C (Boulze et al, 1983; Hubbard et al, 1984), the fluid consumption would most likely increase (Boulze et al, 1983; Hubbard et al, 1984; Sandick et al, 1984; Engell and Hirsch, 1999), which would even further reduce the fluid loss in the normohydrated group.

The concept of tolerable dehydration must, however recognize that dehydration reduces heat dissipation by reducing skin blood flow, which usually results in an increased body core temperature (Coyle and Montain, 1992a,b; Gonzalez-Alonso et al., 1995). Furthermore, as dehydration decreases the stroke volume, cardiovascular strain increases; if dehydration is associated with physical activity and/or heat exposure, cardiovascular strain is further increased. Thus, most people who exercise in a warm environment and experience significant dehydration, will at the same time experience a significant increase in body core temperature, above that experienced during similar exercise conditions when euhydrated (Montain and Coyle, 1992;

Gonzalez-Alonso et al., 1998, 1999; Gonzalez- Alonso, 1998). Coyle (2004) therefore suggested that people might tolerate body water losses of up to 2 % of body mass without significant risk to physical well-being, but just when the environment is cold (5 to 10°C) or temperate (21 to 22°C); however, when people are exposed to a hot environment (> 30°C) and heat exposure is associated with physical activity, dehydration by 2 % of body mass was suggested to impair absolute power production and predispose individuals to heat injury.

Namely, it has been reported (Gonzalez-Alonso, 1998) that a 4 % dehydration without hyperthermia can reduce stroke volume by 7 to 8 % and that hyperthermia without dehydration can also reduce stroke volume by 7 to 8 %. The combination of a 4 % dehydration and hyperthermia, however, elicits synergistic effects, as it reduces stroke volume by more than 20 % (Coyle, 2004). It is therefore expected that prolonged exposure to combined heat stress and dehydration (although mild levels), such as most likely experienced during heat waves, will inevitably cause medical problems; this suggestion is indirectly supported by the observation of increased mortality rates during heat waves (Donaldson et al., 2001; Pirad et al., 2005;).

Finally, although the findings of the present study relate to moderate environment and moderate levels of exercise, it seems reasonable to assume, that the rather small fluid losses observed in the normohydrated group in the present study would accumulate over time, if the exposure to exercise or heat was prolonged, as for example, during heat waves. As the incidence of heat waves is expected to increase in near future because of global warming, the detection of heat related problems, such as the early signs of dehydration seems to become an important issue in general health care in the future.

6. SUMMARY AND CONCLUSIONS

The humanity is faced with a serious challenge of the global warming and consecutively increased ambient temperatures, which affect human performance, well being and health. Dehydration has been recognized as one of the most common heat-related problems and can be critical to human health if it is not detect at its early stage. Despite numerous investigations, no standard method for the assessment of dehydration has yet been recommended. The present study therefore compared five different existing methods, by which one may identify early signs of dehydration.

The first part of the present study was performed in the laboratory were different measurements, including body mass, specific gravity of urine, haemoglobin concentration and hematocrit, as well as classical bioelectrical impedance, and BIA vector analysis were performed on sixteen subjects. The second part of the study was performed outdoors, where the subjects exercised in a moderate environment, with one group of the subjects, i.e. the normohydrated group, ingesting water *ad libitum* and the other one, i.e. the dehydrated group, restricted from drinking. Finally, the last part of the study was again performed in the laboratory, where the same measurements as prior to the exercise were repeated. The aim of the study was to determine, which of the described methods is the most appropriate for detecting mild dehydration in humans and what was the fluid replenishment capacity of the subjects.

According to the results of the present study, mass measurements seem to be one the most reliable methods to detect mild dehydration levels. However mass measurements have to be performed before and after an experimental procedure and the effects of fat and glycogen metabolism on body mass must be taken into account, which somewhat limits the usefulness of the method.

Apart from mass measurements, the measurements of urine specific gravity were also sensitive enough to detect mild changes in hydration level of the subjects. Namely, despite the fact that Usg remained within the limits of normohydration in the normohydrated group, Usg was still significantly increased after the walk in both, the dehydrated and the normohydrated group, which demonstrates that measurements of

Usg can be successfully used to identify even mild changes in hydration status. The assessment of Usg changes can be even further improved if the somewhat subjective colour readings are replaced by the use of refractometers.

The results of the study further demonstrate that assessing relative changes in plasma volume is inappropriate for daily use and that the measurements can be severely confounded by posture and time to the dehydration-inducing condition. It also seems that due to several confounding factors, monitoring relative changes in plasma volume is a less sensitive method to detect mild levels of dehydration than monitoring changes in body mass and USG.

It is suggested that in spite of accounting for several existing precise instructions on how to perform bioelectrical impedance measurements in humans (Heyward and Stolarczyk, 1996; Piccoli, 2005; Buzzell et al, 2007), with some of them specifically aimed to the assessment of hydration status (Piccoli et al, 1994), bioelectrical impedance methods, both classical and BIA-vector, do not provide accurate measurements of body fluid compartments with dehydration and are thus inappropriate for the assessment of hydration status. The summary of the results including advantages and disadvantages of a particular method is presented in Table 7. According to the hypotheses presented in Chapter 2.7, and the results of the present study, the H0a, stating that all methods used for dehydration assessment in the present study will detect dehydration, is rejected.

Lastly, the study also assessed the fluid replenishment capacity of the subjects. The results of the study suggest that although some level of fluid loss is observed when water is available and *ad-libitum* water ingestion allowed during moderate exercise in a moderately warm environment, fluid loss does not exceed 2 %. According to Sawka and Montain (2000) such fluid loss falls within the range of normal hydration. Hypothesis H0b stated that hydration status can be adequately maintained with *ad libitum* water ingestion during physical activity in a moderate-temperature environment; thus as the observed fluid loss was less than 2 %, H0b can not be rejected. It is possible, however, that this result can not be directly translated to the conditions of vigorous exercise and/or severe or prolongued heat exposure. If a

relatively mild dehydration of 1 to 2 % of body mass occurs frequently, which is most likely experienced during heat waves, dehydration can become chronic (Casa 1999) and water deficit may increase, which may predispose individuals to heat related health problems.

In conclusion, it is rather difficult to estimate the complex impacts of global warming and the adjoining climate change on human health. Although in the past science has provided several information about the impacts of climate change on human health and adaptation potential, there are still several questions to be answered. It is expected that global warming will increase global surface temperatures for around 2°C during the next half of the century (Keatinge et al, 2000; Houghton et al, 2001) and while physiological acclimatization to such a change in ambient temperature can take place relatively quickly, the efficient changes in human behaviour to protect against heat waves and the associated prolonged heat exposure are likely to be much slower. Therefore, prevention and adaptation strategies are needed to cope with the consequences of global warming that are already unavoidable due to past emissions of greenhouse gasses. It thus seems reasonable to recommend the establishment of public health programs aimed to inform and help people on how to adjust and defend against heat waves. The results of the present study can, to a certain degree, be used in such an educational program in which special attention should be placed on the elderly, children, the poorest populations, people suffering from predisposing chronic medical conditions, overweight people, and people who work and perform endurance activities in a climatically stressful environment and to specific occupations, such as military service, policemen, farmers, sportsmen, etc. who will inevitably be faced with higher environmental temperatures. It therefore seems that in the future, collaboration between meteorological agencies and health authorities will become indispensable for the prevention of weather-associated risks to human health and well being.

Table 7: *Advantages and disadvantages of the evaluated methods for dehydration assessment*

Method	Advantages	Disadvantages
Body mass measurements	- easy to perform - non-invasive - inexpensive - fast - appropriate for self-monitoring - sensitive to small changes in hydration status	- standardized conditions required - baseline data needed - certified scales required - readings can be affected by confounding factors such as glycogen and fat oxidation when dehydration is associated with exercise
Blood sampling - determination of changes in plasma volume	- precise if performed in standardised conditions and immediately after physical activity	- invasive - require skilled personal - rather expensive - severely affected by body posture - severely affected by a time lag between a given activity and blood sampling
Urine specific gravity determination	- easy to perform - non-invasive - relatively inexpensive - fast - appropriate for self monitoring - guidelines for baseline data exist - sensitive to small changes in hydration	- colour assessment my be subjective, but the readings can be improved with the use of refractometers
Classical bioelectrical impedance	- easy to perform - non-invasive - inexpensive	- completely inappropriate for dehydration assessment due to many confounding factors - unable to detect up to 3 % dehydration and likely even a more severe dehydration
BIA - vector method	- easy to perform - non-invasive - inexpensive	- completely inappropriate for dehydration assessment due to many confounding factors - unable to detect up to 3 % dehydration and likely even a more severe dehydration

7. ASSUMPTIONS, LIMITATIONS, DELIMITATIONS

To perform the present study, some assumptions had to be made and some limitations faced; these are presented below.

The subjects that participated in the study were young, healthy individuals and were therefore not representative for the whole population, which is usually exposed to heat waves. However, the comparison of the methods for the assessment of dehydration did not require the subjects to be enrolled from the complete spectrum of the population.

It was assumed that mass loss of 100 g equalled to water loss of 100 ml. In reality, when dehydration is associated with exercise, some of the observed mass loss must be attributed to glycogen and fat oxidation.

No effort has been made to discern between sweat evaporation, insensible perspiration, and respiratory water loss, which all contributed to the observed water loss after the walk, because these parameters were not relevant for the comparison of different methods for the assessment of hydration status.

The study was performed on two consecutive days outdoors, so that ambient temperature during the walk could not have been identical in both days. It is likely that somewhat different ambient temperature affected the rate of sweating; however the sweating rate could not have been controlled for, as it depends not only on the environmental temperatures, but also on individual characteristics. On each day of the study, however, half of the subjects ingested water *ad libitum* and the other half was restricted from drinking.

Furthermore, the present study was performed in an environment of moderate ambient temperature and the protocol involved exercise of moderate intensity for a relatively short (3-hour) period of time. As ambient temperatures during heat waves can far exceed those of the present study, and prolonged exposure to heat is likely to produce a cumulative effect on water loss, it does not seem unreasonable to assume that the levels of dehydration would be increased, should the experiment be performed in a warmer environment, for a longer period of time and/or with increased exercise intensity. Nevertheless, the main conclusions of the

study are expected to remain the same. Even more, according to some reports (Popowski et al., 2001), during prolonged dehydration, urinary indices may identify changes in hydration status even more accurately than during acute dehydration.

Last, but not least, the speed of walking (≈ 5.5 km/h) used in the present study was similar for all subjects. As the fitness level of the subjects was not identical, the selected speed of walking did not present similar work intensity for all individuals. However, as the main purpose of the study was to induce various levels of dehydration between the two experimental groups, the work intensities during the walk were of secondary importance to this study and therefore were not controlled for.

8. REFERENCES

Adam P. 1997. Evaluation and management of diabetes insipidus. American Family Physician. 55:2146-2153

Alberdi JC. Diaz J. Montero JC. Miron I. 1998. Daily mortality in Madrid Community (Spain) 1986–1991: relationship with atmospheric variables. International Journal of Biometeorology. 14:571-578

Arnfield AJ. 2003. Two decades of urban climate research: a review of turbulence. exchanges of energy and water. and the urban heat island. Intern. Journal of Climatology. 23:1-26

Åstrand PA, Rodahl K, Dahl HA, Stromme SB. 2003. Textbook of Work Physiology: Physiological Bases of Exercise (Hardcover). 4th edition. Champaign (IL): Human Kinetics Publishers. 649 p

Barr S and Costill D. 1989. Water: can the endurance athlete get too much of a good thing? Journal of the American Dietetic Association. 89. 1629-1632,1635

Barrow MW, MD, Clark KA. DO. 1998. Heat-Related Illnesses. American Family Physician. Wright State University. School of Medicine. Dayton. Ohio. 58(3): 759 p.

Bass S, Inge K. 2001. Thermoregulation in young athletes exercising in hot environments. International Sport Med Journal. 2(5):1-6

Battistini N, Virgili F, Bedogni G, Gambella GR, Bini A. 1993. In vivo total body water assessment by total body electrical conductivity in rats suffering perturbations of water compartment equilibrium. British Journal of Nutrition. 70:433-438

Baumgartner RN. 1996. Electrical impedance and total body electrical conductivity. In Roche AF. Heymsfield SB. Lohman TG eds. Human body composition. Champaign (IL): Human Kinetics. 79-107

Bear RW. 1996. Body fluid compartments.
http://www.ursa.kcom.edu/Department/LectureNotes/Spring/BodyFluid.doc (9.1.2008)

Beaumont WV, Young HL, Greenleaf JE. 1974. Plasma fluid and blood constituent shifts during heat exposure in resting men. Aerosp.Med. 45:176-181

Berg J, Tymaczko JL, Stryer L, Clarke ND. 2001. Biochemistry, fifth edition. Editor: Susan Moran. http://www.ncbi.nlm.nih.gov/books/bv.fcgi?indexed=google&rid=stryer.chapter.2911 (18.9.2007)

Boulze D, Montastruc P and Cabanac M. 1983. Water intake, pleasure and water temperature in humans. Physiology and Behavior 30:97-102

Broad EM, Burke LM, Gox GR, Heeley P and Riley M. 1996. Body weight changes and voluntary fluid intakes during training and competition sessions in team sports. International Journal of sports nutrition. 6:307-320

Brück K. 1989. Thermal balance and the regulation of body temperature. Page 627. In: Schmidt R.F. and Thews G eds. 1989. Human Physiology. 2nd ed. Berlin, Heidelberg, New York: Springer Verlag, 825 p.

Buskirk ER, Iampietro PF, Bass DE. 1958. Work performance after dehydration: effects of physical conditioning and heat acclimatization. Journal of Applied Physiology.12:189-194

Buzzell PR, BS, Pintauro SJ. 2007. Bioelectrical impedance analysis. An interactive tutorial. Department of nutrition and food sciences. University of Vermont
http://nutrition.uvm.edu/bodycomp/bia/ (19.6.2007)

Campbell NA. 1987. Biology. 3rd ed. California: Benjamin Cummings. 790 p.

Cadarette BS, Sawka MN, Toner MM, Pandolf KB. 1984. Aerobic fitness and the hypohydration response to exercise-heat stress. Aviation Space Environmental Medicine. 55:507-512

Carter TR, Hulme M, Crossley JF, Malyshev S, New MG, Schlesinger ME, Tuomenvirta H 2000. Climate Change in the 21st Century: Interim Characterizations based on the New IPCC Emissions Scenarios. The Finnish Environment 433. Finnish Environment Institute. Helsinki. Page 150

Casa DJ. 1999. Exercise in the Heat. II. Critical Concepts in Rehydration. Exertional Heat Illnesses. and Maximizing Athletic Performance. Journal of Athletic Training. 34(3):253-262

Cegnar T. 2004. Climate change and human health and well-being. Geografski vestnik. 77-1: 79-88

Cheuvront SN, Sawka MN. 2005. Hydration Assessment of Athletes. Sports Science Exchange. 18(2):1-12

Convertino VA, Armstrong LE, Coyle EF, Mack GW, Sawka MN, Senay LC Jr, Sherman WM. 1996. American College of Sports Medicine position stand. Exercise and fluid replacement. Medicine Science Sports and Exercise. 28(10):i-ix

Costil DL and Fink WJ. 1974. Plasma volume changes following exercise and thermal dehydration. Journal of Applied Physiology. 37:521-525

Costill DL, Cote R, Miller E, Miller T, Wynder S. 1975. Water and electrolyte replacement during repeated days of work in the heat. Aviation, Space, and Environmental Medicine. 46:795–800

Coyle EF and Montain SJ. 1992a. Benefits of fluid replacement with carbohydrate during exercise. Medicine and Science in Sports and Exercise. 24:S324–S330

Coyle EF and Montain SJ. 1992b. Carbohydrate and fluid ingestion during exercise: are there trade-offs? Medicine and Science in Sports and Exercise. 24:671–678

Coyle EF. 2004. Fluid and fuel intake during exercise. Journal of sports science. 22:39-55

Davis RE, Knappenberger PC, Michaels PJ, Novicoff WM. 2003. Changing heat-related mortality in the United States. Environmental Health Perspectives. 111(14):1712–1718

De Lorenzo A, Deurenberg P, Andreoli A, Sasso GF, Palestini M, Docimo R. 1994. Multifrequency impedance in the assessment of body water losses during dialysis. Renal Physiology & Biochemistry. 17:326–32

Dill DB and Costill DL. 1974. Calculation of percentage change in volumes of blood, plasma, and red cells in dehydration. Journal of Applied Physiology. 37(2):247-248

Donaldson G, Kovats RS, KeatingeWR, McMichael AJ. 2001. Overview of climate change impacts on human health in the UK. In: Maynard RL, ed. Health effects of climate change in the UK. London: Department of Health, pp. 70-80

EEA. 2004. Impacts of Europe's changing climate. An indicator-based assessment. Copenhagen; 2:2-33

EEA. 2005. Environment and health. Report. Copenhagen; 10:4-9

Eisman L. 1972. Biology and Human Progress. Englewood Cliffs, NJ: Prentice Hall. 125 p.

Engell D and Hirsch E 1999. Environmental and sensory modulation of fluid intake in humans. In: Thirst: Physiological and Psychological Aspects, Ramsey DJ and Booth DA eds. Berlin: Springer-Verlag, pp. 382-402

Ferguson HS. 2002. Management of heat related illness enhanced by climate change and global warming in the North West Kimberley region of Western Australia. Journal of Rural and Remote Environmental Health. 1(1):13-18

Fortney SM, Nadel ER, Wenger CB, Bove JR. 1981. Effect of acute alteration of blood volume on circulatory performance in humans. Journal of Applied Physiology. 50:292-298

Glossary of terms for thermal physiology. 2001. The Japanese Journal of Physiology. 51(2):245

Gonzalez-Alonso J, Mora-Rodriguez R, Below PR and Coyle EF. 1995. Dehydration reduces cardiac output and increases systemic and cutaneous vascular resistance during exercise. Journal of Applied Physiology. 79:1487–1496

Gonzalez-Alonso J, Calbet JAL and Nielsen B. 1998. Muscle blood flow is reduced with dehydration during prolonged exercise in humans. Journal of Applied Physiology. 513:895–905

Gonzalez-Alonso J. 1998. Separate and combined influences of dehydration and hyperthermia on cardiovascular responses to exercise. International Journal of Sports Medicine. 19(suppl.2):S111–S114

Gonzalez-Alonso J, Teller C, Andersen SL, Jensen FB, Hyldig T and Nielsen B. 1999. Influence of body temperature on the development of fatigue during prolonged exercise in the heat. Journal of Applied Physiology. 86:1032–1039

Greenleaf JE. 1992. Problem: thirst, drinking behaviour, and involuntary dehydration. Medicine Science Sports and Exercise. 24:645–656

Hagan RD, Diaz FJ and Horvath SM. 1978. Plasma volume changes with movement to supine and standing positions. Journal of Applied Physiology. 45:414-418

Hales JRS, Hubbard RW, Gaffin SL. 1996. Limitations of heat tolerance. In: Fregly MJ, Blatteis CM, eds. Handbook of physiology. Section 4. New York: Oxford University Press, pp. 285-355

Hargreaves M, Dillo P, Angus D and Febbraio M. 1996. Effect of fluid ingestion on muscle metabolism during prolonged exercise. Journal of Applied Physiology. 80:363-366

Harrison MH, Edwards RJ, Fennessy PA. 1978. Intravascular volume and tonicity as factors in the regulation of body temperature. Journal of Applied Physiology. 44(1):69-75

Harrison MH. 1985. Effects on thermal stress and exercise on blood volume in humans. Physiology Rev. 65(1):149-209

Hassanein T, Razack A, Gavaler J, Van Thiel DH. 1992. Heatstroke: its clinical and pathological presentation. with particular attention to the liver. Am J Gastroenterol. 87:1382-9

Heyward VH and Stolarczyk LM. 1996. Applied Body Composition Assessment. 3:44-55

Houghton JT, Ding Y, Griggs DJ, Noguer M, Linden PJ, Dai X, Maskell K, Johnson CA 2001. IPCC Climate change 2001. The scientific basis. Cambridge University Press. Cambridge. United Kingdom

Hubbard RW, Maller O, Sawka MN, Francesconi RN, Drolet L, and Young AJ. 1984. Voluntary dehydration and alliesthesia for water. Journal of Applied. Physiology. 57:868-875

Hughes L. 2000. Biological consequences of global warming: is the signal already. Tree. 15(2):56-61

Intergovernmental Panel on Climate Change (IPCC). 2001. Climate Change 2001: Synthesis Report. Summary for Policymakers. IPPC Third Assessment Report. Wembley. United Kingdom, pp. 5-6

Intergovernmental Panel on Climate Change (IPCC). 2007a. Climate Change 2007: The Physical Science Basis. Summary for Policymakers. Contribution of Working Group I to the Fourth Assessment Report of the IPCC. Paris, France. 16 p.
http://www.ipcc.ch/SPM2feb07.pdf (17.7.2007)

Intergovernmental Panel on Climate Change (IPCC). 2007b. Climate Change 2007: Climate Change Impacts, Adaptation and Vulnerability. Summary for Policymakers. IPCC Working Group II Fourth Assessment Report

http://www.ipcc.ch/SPM6avr07.pdf (18.7.2007)

Intergovernmental Panel on Climate Change (IPCC). 2007c. Climate Change 2007: Mitigation. Contribution of Working Group III to the Fourth Assessment Report of the IPCC. Cambridge. UK and NY, pp. 3

Jackson AS, Pollock ML. 1985. Practical assessment of body composition. The Physician and Sportsmedicine. 13:76-90

Jebb SA, Cole TJ, Doman D, Murgatroyd PR, Prentice AM. 2000. Evaluation of the novel Tanita body-fat analyser to measure body composition by comparison with a four-compartment model. British Journal of Nutrition. 83:115–22

Kajfež-Bogataj L. 2005. Climate change impacts on quality of human life. Acta agriculturae Slovenica. 85(1):41-54

Kalkstein LS, Smoyer KE. 1993. The impact of climate change on human health: some international implications. Experientia. 49:969–979

Kavouras SA. 2002. Assessing hydration status. Current Opinion in Clinical Nutrition and Metabolic Care. 5:519-524

Keatinge WR, Donaldson GC, Cordioli E, Martinelli M, Kunst AE, Mackenbach JP, Nayha S, Vuori I. 2000. Heat related mortality in warm and cold regions of Europe: observational study. BMJ. 312:670-673

Keatinge WR. 2003. Death in heat waves. BMJ 327:512-513

King J. 2004. Thermoregulation: Physiological Responses and Adaptations to Exercise in Hot and Cold Environments. Journal of Hyperplasia Research, 4
http://www.abcbodybuilding.com/magazine04/thermoregulation.htm (12.2.2007)

Knochel JP. 1996. Clinical complications of body fluid and electrolyte imbalance. In: Buskirk ER, Puhl SM, eds. Body fluid Balance Exercise and Sport. New York, NY: CRC Press, pp. 297-317

Koppe C, Kovats S, Jendritzky G, Menne B. 2004. Heat – waves risk and responses. WHO. Health and Global Environmental Change. 2:15-25

Koulmann N, Jimenz C, Regal D, Bolliet P, Launay JC, Savourey G, Melin B. 2000. Use of bioelectrical impedance analysis to estimate body fluid compartments after acute variations of the body hydration level. Medicine & Science in Sports & Exercise. 32(4):857-864

Kyle UG, De Lorenzo IBAD, Deurenberg P, Elia M, Gomez JM, Heitmann BL, Kent-Smith L, Melchior J, Pirlich M, Scharfetter H, Schols AMWJ and Pichard C. 2004a. Composition of the ESPEN Working Group. Bioelectrical impedance analysis part I: review of principles and methods. Clinical Nutrition. 23: 1226–1243

Kyle UG,, De Lorenzo IBAD, Deurenberg P, Elia M, Gomez JM, Heitmann BL, Kent-Smith L, Melchior J, Pirlich M, Scharfetter H, Schols AMWJ, Pichard C. 2004b. Bioelectrical impedance analysis part II: utilization in clinical practice. Clinical Nutrition. 23:1430-1453

Kramer GC, English TP, Gunther RA, Holcroft JW. 1989. Physiological mechanism of fluid resustication with hypertonic/Hyperoncotic solutions. In: Passmore JC ed. Perspectives in Shock Research: Metabolism, Imunology, Mediators, and Models. New York, NY: Alan RL, Inc. 311

Krane EJ. 1996. Diabetic Ketoacidosis and Cerebral Edema. Departments of Pediatrics and Anesthesiology. Stanford University Medical Center
http://pedsccm.org/FILE-CABINET/Metab/DKA-CEdema.html#krane#krane (26.11.2007)

Kushner RF. 1992. Bioelectrical impedance analysis: a review of principles and applications. Journal of American. College. Nutrition. 11:199–209

Ladell W. 1955. The effects of water and salt intake upon the performance of men working in hot and humid environments. Journal of Physiology. 127:11–26

Larsen J. 2006. Setting the record straight: More than 52000 Europeans Died from Heat in Summer 2003. Earth policy institute. http://www.earth-policy.org/Updates/2006/Update56.htm#top (9.2.2007)

Lennert T and Luft FC. 1997. Kidney on vacation: the notion of renal work and the introduction of nonpharmological therapies. American Journal of kidney diseases. 29:777-780

Lippincott WW. 2005. Instructor's resource CD-ROM to accompany Porth′s Pathophysiology: Concept of altered health states. Seventh edition
http://connections.lww.com/Products/porth7e/documents/Ch33/pdf/33_004.pdf (12.12.2007)

Mack GW, Nadel ER. 1996. Body fluid balance during heat stress in humans. In: Fregly MJ, Blatteis CM, eds. Environmental physiology. New York: Oxford University Press, pp. 187-214

Marriott BM. 1993. Nutritional needs in hot environments: application for military personnel in field operations. Washington, DC: National Academy Press. 378 p.

Martinez-Nevarro F, Simon-Soria F, Lopez-Abente G. 2004. Evaluation of the impact of the heat wave in the summer of 2003 on mortality. Gaceta Saintaria. (18):185-189

Maughan RJ, Leiper JB, Shirreffs SM. 1996. Restoration of fluid balance after exercise-induced dehydration: effects of food and fluid intake. European Journal of Applied Physiology and Occupational Physiology. 73:317–325

McArdle WD, Katch FI, Katch VL. 2001. Exercise Physiology. Energy. Nutrition and Human Performance. Lippincott Williams & Wilkins. 5 edition. 1158 p.

McCarthy JJ, Canziani OF, Leary NA, Dokken DJ, White KS. 2001. Climate change 2001: Impacts. Adaptation. and Vulnerability. Contribution of Working Group II on the Third

Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge. United Kingdom. Available at: http://www.grida.no/climate/ipcc_tar/wg2/index.htm (19.11.2006)

Mitchell JW, Nadel RE, Stolwijk JA. 1972. Respiratory weight losses during exercise. Journal of Applied Physiology. 32:474-476

Mitchell JFB, Johns TC, Gregory JM, Tett SFB. 1995. Climate response to increasing levels of greenhouse gases and sulphate aerosols. Nature. 376(6540):501-504

Montain SJ and Coyle EF. 1992 .Influence of graded dehydration on hyperthermia and cardiovascular drift during exercise. Journal of Applied Physiology. 73:1340-1350

Montain SJ, Maughan RJ, Sawka MN. 1996. Fluid replacement strategies for exercise in hot weather. Athletic Therapy Today. 1:24-7

Mrak M, Hočevar G, Zagrajšek J, Šinkovec M, Adamič M, Honn R, Živko A, Kovačič I. 2006. Dnevnik tekača. 3:7-11

Nadel ER. 1984. Body fluid and electrolyte balance during exercise: competing demands with temperature regulation. In: Thermal Physiology, Hales JRA ed. New York; pp. 365-376

National Collegiate Athletic Association Wrestling Rules Committee. Indianapolis. 1998. In: National Collegiate Athletic Association. 1998–1999 wrestling weight certification program

Noakes TD. 2003a. Overconsumption of fluids by athletes. British Medical Journal. 327(7407):113-114

Noakes TD. 2003b. Lore of Running. Leeds. Human Kinetics. 931 p

Nose H, Mack GW, Shi X, Nadel ER. 1988. Shift in body fluid compartments after dehydration in humans. New Haven. Connecticut. 65:318-323

Nunneley SA and Stribley RF. 1979. Heat and acute dehydration effects on acceleration response in man. Journal of Applied Physiology. 47:197-200

O'Brien C, Young AJ, Sawka MN. 2002. Bioelectrical impedance to estimate changes in hydration status. International Journal of Sports Medicine. 23:361-366

Oke TR. 1997. Urban climates and global environmental change. In: Thompson RD. Parry AH. eds. Applied climatology: principles and practice. London: Routledge, pp. 273-287

Oppliger RA, Bartok C. 2002. Hydration Testing of Athletes. Sports Med. 32(15):959-971

Piccoli A, Rossi B, Pillon L, Bucciante G. 1994. A new method for monitoring body fluid variation by bioimpedance analysis: the RXc graph. Kidney International. 46:534-539

Piccoli A, Pillon L, Favaro E. 1997. Asymmetry of the total body water prediction bias using the impedance index. Nutrition. 13:438-441

Piccoli A. 2005. Whole body – single frequency bioimpedance. In: Ronco C. Brendolan A. Levin NW. (Eds.): Cardiovascular disorders in haemodialysis. Contrib Nephrol. Basel. Karger. 149:150-161

Pirad P, Vandentorren S, Pascal M, Laaidi K, Le Tertre a, Cassadou S, Ledrans M. 2005. Summary of the mortality impact assessment of the 2003 heat wave in France. Euro Surveillance; 10(7):153-6

Pitts GJ, and Consolazio FC. 1944. Work in the heat as affected by intake of water, salt and glucose. American Journal of Physiology. 142:253-259

Popowski LA, Oppliger RA, Lambert GP, Johnson RF, Johnson AK and Gisolfi CV. 2001. Blood and urinary measures of hydration status during progressive acute dehydration. Medicine and science in sports and exercise. 33(5):747-753

Purdy K. 2000. Howard Hughes Medical Institute. Available at:

http://www.hhmi.org/cgi-bin/askascientist/highlight.pl?kw=&file=answers%2Fgeneral%2Fans_016.html (9.2.2007)

Pradella M, Dorizzi RM, Rigolin F. 1988. Relative density of urine: methods and clinical significance., Critical reviews in clinical laboratory sciences 26:195-242

Saltin B. 1964. Circulatory response to submaximal and maximal exercise after thermal dehydration. Journal of Applied Physiology. 19:1125-1182

Sandick BL, Engell DB and Maller O. 1984. Perception of water temperature and effects for humans after exercise. Physiology and Behavior. 32:851-855

Sawka MN, Toner MM, Francesconi RP, Pandolf KB. 1983. Hypohydration and exercise: effects of heat acclimation, gender, and environment. Journal of Applied Physiology. 55:1147-1153

Sawka MN. 1988. Body fluid responses and hypohydration during exercise heat stress. In: Pandolf KB, Sawka MN, Gonzalez RR, eds. Human performance physiology and environmental medicine at terrestrial extremes. Indianapolis: Cooper Publishing Group, pp. 227-266

Sawka MN, Pandolf KB. 1990. Effects of body water loss on physiological function and exercise performance. In: Gisolfi CV, Lamb DR, eds. Perspectives in exercise science and sports medicine. Vol 3. Carmel, Benchmark Press, pp 1-38

Sawka MN. 1992. Physiological consequences of hypohydration: exercise performance and thermoregulation. Medicine Science Sports and Exercise. 24:657-70

Sawka MN, Montain SJ. 2000. Fluid and electrolyte supplementation for exercise heat stress 1-4. American Journal of Clinical Nutrition. 72(suppl): 564S-72S

Sawka MN, Cheuvront SN. Carter R. 2005.l Human water needs. Nutrition Reviews. 63(6):33

Senay LC. 1979. Temperature regulation and hypohydration: a singular view. Journal of Applied Physiology. 47:1-7

Strasinger SK. 1994. Urinalysis and body fluids. Philadelphia (PA): FA Davis Company. 250 p.

Stuempfle KJ. Drury DG. 2003. Comparison of 3 Methods to Assess Urine Specific Gravity in Collegiate Wrestlers. Journal of Athletic training. 38(4):315-319

Szlyk PC, Sils IV, Francesconi RP, Hubbard RW. 1990. Patterns of human drinking: effects of exercise, water temperature, and food consumption. Aviation, Space, and Environmental Medicine. 61:43-48

UCLA. 2004. Department of statistics. University of California. Los Angeles. California. United States of America. http://calculators.stat.ucla.edu/powercalc (31.3 2006)

US Environmental Protection Agency. 2001. Greenhouse effect schematic. http://www.epa.gov/climatechange/kids/greenhouse.html (2.10.2006).

Utter AC, Nieman DC, Ward AN, Butterworth DE. 1999. Use of the leg-to-leg bioelectrical impedance method in assessing body-composition change in obese women. American Journal of Clinical Nutrition. 69:603–7

van Loan MD.1990. Bioelectrical impedance analysis to determine fat-free mass, total body water, and body fat. American Journal of Sports Medicine. 10:205–217

van Loon LJ, Greenhaff PL, Constantin-Teodosiu D, Saris WH, Wagenmakers AJ. 2001. The effects of increasing exercise intensity on muscle fuel utilisation in humans. The Journal of Physiology. 536:295-304

Wang ZM. Pierson RN. Heymsfield SB. 1992. The five-level model: a new approach to organizing body-composition research. American Society for Clinical Nutrition. 56:19-28

Weinberg AD. Minaker KL. 1995. Dehydration: evaluation and management in older adults. Journal of the American Medical Association. 274:1552-1556

WHO. 1984. http://www.great-lakes.net/humanhealth/about/words_h.html (29.3.2007)

WHO. WMO. UNEP. 2003. Climate change and human health – Risk and Responses. Summary. McMichael AJ. WHO. Switzerland, pp. 11

Wyndham C and Strydom N. 1969. The danger of an inadequate water intake during marathon running. South African Medical Journal. 43:893–896

Yannakoulia M, Kerampoulos A, Tsakalakos N and Matalas AL. 2000. Body composition in dancers: the bioelectrical impedance method. Medicine and science in sports and exercise. 32(1):228-234

9. APPENDICES

Appendix A

Individual values of age (years), height (cm) and mass (kg) before and after the walk, mass loss during the experiment (kg), percent change in body mass (%), and water consumption (g) during the walk for the normohydrated and dehydrated group.

Subjects/term	Age (years)	Height (cm)	Mass before (kg)	Mass after (kg)	Mass loss (kg)	Percent change in body mass (%)	Water consumption during the walk (g)
Normohydrated group							
male	21	183,8	76,8	75,9	0,9	1,2	1066
male	23	186,4	76,4	75,5	0,9	1,2	1069
female	20	165,3	61,5	61,1	0,4	0,7	1160
female	19	177,8	57,9	57,7	0,2	0,3	802
male	30	181,1	85,5	84,4	1,1	1,3	994
male	26	181,5	73,7	73	0,7	0,9	1527
female	19	162,1	56,4	56,2	0,2	0,4	986
female	24	171,5	78,1	77,7	0,4	0,5	1149
AVERAGE	23	176	70,8	70,2	0,6	0,8	1094
SD	4	11	12,9	12,6	0,8	1,0	208
Dehydrated group							
male	26	184,9	84,9	82,8	2,1	2,5	0
male	26	181,6	69,9	67	2,9	4,1	0
female	22	160,2	47,5	45,9	1,6	3,4	0
female	30	175,9	69,7	68,3	1,4	2,0	0
male	18	182	68,2	67,5	0,7	1,0	0
male	23	180,2	80,5	78,3	2,2	2,7	0
female	28	168,9	55,6	54,4	1,2	2,2	0
female	24	180,2	69,8	68,5	1,3	1,9	0
AVERAGE	25	177	68,6	67,0	1,6	2,3	0
SD	4	9	14,0	13,8	1,0	1,5	0

Appendix B

Individual values of haemoglobin (Hb; g/L), hematocrit (Hct), the calculated plasma volume change (PV change; %), and plasma sodium (Na^+; mmol/L) before and after the walk, for the normohydrated and dehydrated group.

Subjects/term	**Hb before (g/L)**	**Hb after (g/L)**	**Hct before (L)**	**Hct after (L)**	**PV change (%)**	Na^+ before **(mmol/L)**	Na^+ after **(mmol/L)**
Normohydrated group							
male	139	132	0,42	0,39	10,75	140	139
male	156	151	0,46	0,44	7,14	141	139
female	130	123	0,38	0,37	7,40	140	139
female	118	109	0,36	0,34	11,64	141	139
male	135	137	0,4	0,41	-3,10	139	139
male	148	139	0,45	0,42	12,28	139	138
female	127	130	0,38	0,4	-5,46	N.A.	N.A.
female	121	115	0,36	0,36	5,22	137	140
AVERAGE	134	130	0,40	0,39	5,73	140	139
SD	13	14	0,04	0,03	6,67	1	1
Dehydrated group							
male	129	122	0,38	0,36	9,15	142	146
male	140	135	0,42	0,41	5,49	142	144
female	130	127	0,4	0,4	2,36	139	140
female	129	127	0,38	0,37	3,21	141	145
male	140	140	0,41	0,41	0,00	139	N.A.
male	138	139	0,41	0,41	-0,72	138	140
female	133	128	0,4	0,38	7,37	136	139
female	126	126	0,38	0,38	0,00	137	137
AVERAGE	133	131	0,40	0,39	3,36	139	142
SD	6	7	0,02	0,02	3,67	2	3

Appendix C

Individual values of urine specific gravity (Usg) before and after the walk for the normohydrated and dehydrated group.

Subjects/term	Usg before	Usg after
Normohydrated group		
male	1,005	1,020
male	1,005	1,030
female	1,010	1,010
female	1,030	1,030
male	1,005	1,015
male	1,015	1,015
female	1,005	1,005
female	1,010	1,020
AVERAGE	1,011	1,018
SD	0,009	0,009
Dehydrated group		
male	1,015	1,030
male	1,010	1,030
female	1,010	1,030
female	1,025	1,030
male	1,020	1,015
male	1,020	1,030
female	1,005	1,030
female	1,015	1,025
AVERAGE	1,015	1,028
SD	0,007	0,005

Appendix D

Individual values of systolic arterial pressure (SAP; mm Hg), diastolic arterial pressure (DAP; mm Hg), and tympanic temperature (Tty; °C) before and after the walk for the normohydrated and dehydrated group.

Subjects/term	SAP before (mm Hg)	SAP after (mm Hg)	DAP before (mm Hg)	DAP after (mm Hg)	Tty before (°C)	Tty after (°C)
Normohydrated group						
male	128	124	70	68	36,8	36,6
male	114	118	72	78	36,7	36,4
female	112	110	68	68	37	36,6
female	119	108	76	74	36,9	36,7
male	110	104	64	60	36,7	36,7
male	130	120	70	68	36,3	36,7
female	108	110	78	72	37,2	36,7
female	122	104	78	66	37,1	37
AVERAGE	118	112	72	69	36,8	36,7
SD	8	8	5	5	0,3	0,2
Dehydrated group						
male	120	118	84	72	37,3	36,5
male	128	116	74	82	37,4	36,9
female	118	110	81	74	36,8	37,1
female	116	110	81	80	37,5	37,5
male	122	118	62	78	36,3	36,8
male	120	110	70	60	36,6	36,7
female	114	106	72	68	37,1	36,8
female	104	60	90	52	37,1	37
AVERAGE	118	106	77	71	37,0	36,9
SD	7	19	9	10	0,4	0,3

Appendix E

Individual values of total body water (TBW; L), intracellular water (ICW; L), body cell mass (BCM; kg) and extra cellular water (ECW; L), both before and after the walk for the normohydrated and dehydrated group, as assessed by classical bioimpedance measurements.

Subjects/term	TBW before (L)	TBW after (L)	ICW before (L)	ICW after (L)	BCM before (kg)	BCM after (kg)	ECW before (L)	ECW after (L)
Normohydrated group								
male	44,6	41,0	27,5	23,5	30,2	25,8	17,1	17,5
male	41,8	42,3	26,2	26,3	28,7	28,9	15,6	16,0
female	30,2	31,1	17,0	17,3	18,6	19,0	13,2	13,8
female	29,3	30,3	17,5	17,6	19,2	19,3	11,8	12,7
male	48,2	47,9	28,3	27,9	31,1	30,6	19,9	20,0
male	42,2	42,6	26,5	26,5	29,0	29,1	15,7	16,1
female	28,9	29,1	17,0	16,9	18,6	18,5	11,9	12,2
female	37,2	37,0	20,9	20,6	23,0	22,6	16,3	16,4
AVERAGE	37,8	37,7	22,6	22,1	24,8	24,2	15,2	15,6
SD	7,6	6,9	5,0	4,6	5,5	5,0	2,8	2,6
Dehydrated group								
male	48,4	48,7	30,1	30,0	33,0	32,9	18,3	18,7
male	39,7	38,6	24,8	23,7	27,2	26,1	14,9	14,9
female	27,6	27,2	16,7	16,2	18,3	17,8	10,9	11,0
female	36,0	36,8	20,5	20,8	22,5	22,8	15,5	16,0
male	40,9	40,4	26,3	26,0	28,8	28,4	14,6	14,4
male	45,7	45,8	28,7	29,0	31,4	31,8	17,0	16,8
female	29,3	29,1	17,1	16,8	18,7	18,4	12,2	12,3
female	36,8	36,9	22,0	21,8	24,1	23,8	14,8	15,1
AVERAGE	38,1	37,9	23,3	23,0	25,5	25,3	14,8	14,9
SD	7,3	7,4	5,0	5,1	5,5	5,6	2,4	2,4

Appendix F

Individual values of resistance (R/h;Ω/m) and reactance (Xc/h; Ω/m) before and after the walk for the normohydrated and dehydrated group, as assessed by classical bioimpedance measurements.

Subjects/term	RZ before (Ohm/m)	RZ after (Ohm/m)	XC before (Ohm/m)	XC after (Ohm/m)
Normohydrated group				
male	291	303	34	33
male	354	335	42	39
female	418	387	43	39
female	506	469	51	44
male	274	273	29	28
male	313	301	37	35
female	418	411	49	46
female	327	330	37	36
AVERAGE	363	352	40	38
SD	79	66	7	6
Dehydrated group				
male	273	259	34	31
male	340	345	39	37
female	407	413	51	48
female	346	326	37	35
male	312	314	38	38
male	287	274	36	35
female	437	439	46	44
female	355	350	42	39
AVERAGE	345	340	40	39
SD	53	62	6	6