

# Thermoregulation and fluid balance during a 30-km march in 60- versus 80-year-old subjects

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**Abstract** The presence of impaired thermoregulatory and fluid balance responses to exercise in older individuals is well established. To improve our understanding on thermoregulation and fluid balance during exercise in older individuals, we compared thermoregulatory and fluid balance responses between sexagenarians and octogenarians during prolonged exercise. Forty sexagenarians (60±1 year) and 36 octogenarians (81±2 year) volunteered to participate in a 30-km march at a self-selected pace. Intestinal temperature ( $T_{in}$ ) and heart rate were recorded every 5 km. Subjects reported fluid intake, while urine output was measured and sweat rate was calculated. Octogenarians demonstrated a lower baseline  $T_{in}$  and a larger exercise-induced increase in  $T_{in}$  compared to sexagenarians (1.2±0.5 °C versus 0.7±0.4 °C,  $p<0.01$ ), while maximum  $T_{in}$  tended to be higher in octogenarians (38.4±0.4 °C versus 38.2±0.3 °C,  $p=0.09$ ). Exercise intensity (70±11 % versus 70±9 %) and exercise duration (7 h 45 min±0 h 57 min versus 7 h 24 min±0 h 58 min) were not

different between octogenarians and sexagenarians. Octogenarians demonstrated lower fluid intake (251±97 mL/h versus 325±125 mL/h,  $p=0.01$ ) and urine output (28±22 mL/h versus 52±40 mL/h,  $p<0.01$ ) compared to sexagenarians. Furthermore, the sweat rate tended to be lower (294±150 mL/h versus 364±148 mL/h,  $p=0.07$ ) in the octogenarian group. Sodium levels and plasma volume changes were not different between sexagenarians and octogenarians (all  $p>0.05$ ). These results suggest that thermoregulatory responses deteriorate with advancing age, while fluid balance is regulated appropriately during a 30-km walking march under moderate ambient conditions.

**Keywords** Walking exercise · Hyperthermia · Dehydration · Aging · Endurance exercise

## Introduction

The increased metabolic heat production during exercise leads to a rise in core body temperature (Bouchama and Knochel 2002; Kenefick et al. 2007) and may ultimately lead to the development of heat-related illnesses (Tattersson et al. 2000). The primary heat dissipating mechanism during exercise is evaporation through sweating, accounting for 80 % of total heat loss (Bar-Or 1998; Sawka et al. 2007). However, the production of sweat in combination with insufficient fluid replacement may lead to the development of dehydration (Cheuvront et al. 2010) and could impair exercise

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performance and increase the risk to develop hyperthermia (Sawka 1992).

Advanced age is associated with a negative impact on thermoregulatory and fluid balance responses during exercise (Blatteis 2012; Chester and Rudolph 2011). Previous studies, which compared young versus old subjects, showed that elderly  $\geq 60$  years have a lower baseline core body temperature, a reduced skin vasodilatory capacity, a less effective sweat response, and a decreased sensitivity of the thermal receptors (Blatteis 2012; Chester and Rudolph 2011; Guergova and Dufour 2011). Furthermore, advanced age is associated with a decreased total body water (Davis and Mnaker 1994; Schoeller 1989), decreased thirst sensation (Mack et al. 1994; Phillips et al. 1984), decline in kidney functioning (Davis and Mnaker 1994; Lindeman et al. 1985), and a reduced plasma vasopressin regulation at rest and during dehydration (Miller and Shock 1953; Sheehy et al. 1999). These characteristics make older humans a vulnerable population for the development of hyperthermia and dehydration (Begum and Johnson 2010; Blatteis 2012; Chester and Rudolph 2011; Mentis 2006; Sheehy et al. 1999). Most of this knowledge is derived from cross-sectional observations between cohorts of young (usually  $<30$  years) and older humans (usually  $>60$  years). Accordingly, it is unknown whether thermoregulation and fluid balance control deteriorates further with aging or plateaus at some point.

The purpose of our study was, therefore, to compare thermoregulatory responses and fluid balance during prolonged, moderate-intensity exercise between sexagenarians and octogenarians. We hypothesized that exercise-induced thermoregulatory responses and fluid balance control are further deteriorated in octogenarians compared to sexagenarians.

## Materials and methods

### Subjects

A total of 79 subjects volunteered to participate in this study (Table 1). Subjects were recruited via a newsletter, which was distributed by the organization of the Nijmegen Four Days Marches (an annual walking event in The Netherlands). Based on the age classification of previous publications, subjects were divided into the group of sexagenarians ( $60 \pm 1$  year, 24 men and 16 women) or octogenarians ( $81 \pm 2$  year, 25 men and 14

women). The following subjects were excluded from participation: I) body mass  $<36.5$  kg, II) implanted electro-medical device, III) a history of gastrointestinal disease or abdominal surgery, and IV) a scheduled MRI-scan within 1 week after participating in the present study (all based on the use of the temperature pill). The study was approved by the Medical Ethical Committee of the Radboud University Medical Center, and all subjects gave written informed consent prior to participation. This study was conducted in accordance with the Declaration of Helsinki.

### Experimental design

Baseline measurements, including subject characteristics, body composition, urine specific gravity and blood levels of sodium, hemoglobin and hematocrit, were performed in our temperature controlled laboratory one or two days prior to the exercise bout. The blood pressure was measured in supine position after lying on a bed for 5 min, while a blood sample was taken in sitting position. Immediately before the start of exercise, subjects underwent assessment of body mass, heart rate (HR), and intestinal temperature ( $T_{in}$ ). Thereafter, subjects participated in the first day of the Nijmegen Four Days marches and walked 30 km at a self-selected pace, all starting at 7:00 AM. The exercise bout consisted of a 30-km self-paces march on a flat course over tarmac roads. During exercise,  $T_{in}$  and HR were recorded after every 5 km, while urine excretion was collected using collecting bags and all subjects registered their fluid intake using a diary. Directly after finishing, subjects reported to our laboratory to determine post-exercise body mass, HR,  $T_{in}$ , fluid balance (urine and blood analysis).

### Measurements

*Subject characteristics* Body mass (Seca 888 scale, Hamburg, Germany) and body height were measured, and body mass index (BMI) was calculated. A four-point skinfold thickness measurement (biceps, triceps, sub-scapular, supra-iliac) was performed in order to calculate the lean body mass (Durnin and Womersley 1974). Waist circumference was measured midway between the lower rib margin and iliac crest. Hip circumference was measured at the level of widest circumference over greater trochanters. Waist-to-hip ratio was calculated as waist

**Table 1** Subject characteristics

Parameter	Sexagenarians ( <i>n</i> =40)	Octogenarians ( <i>n</i> =36)	<i>P</i> value
Demographic characteristics			
Age (year)	60±1	81±2	<0.01
Height (cm)	172±10	168±9	0.08
Weight (kg)	81.5±15.5	68.3±11.3	<0.01
Body-mass index (kg/m <sup>2</sup> ) <sup>a</sup>	27.4±3.8	24.0±2.6	<0.01
Body fat (%)	34.0±5.8	28.2±6.9	<0.01
Lean body mass (kg)	54.0±10.9	49.0±9.1	0.04
Abdominal circumference (cm)	96.4±13.1	89.2±10.4	0.01
Men	102.3±12.8	94.4±8.6	0.02
Women	87.6±7.7	79.8±5.5	<0.01
Waist circumference (cm)	100.9±7.3	95.4±7.1	<0.01
Waist-hip ratio	0.96±0.09	0.94±0.09	0.38
Health status			
Physical activity			
Exercise (h/week) <sup>a</sup>	3.1±2.9	4.8±4.5	0.29
Training status (km/year) <sup>a</sup>	501±423	990±723	<0.01
Mean arterial pressure (mmHg)	102±12	103±12	0.62
Rest heart rate (bpm)	67±12	67±16	0.95
Pathology			
Cardiovascular diseases ( <i>n</i> (%))	6 (15.0 %)	4 (11.1 %)	0.62
Hypertension ( <i>n</i> (%))	15 (37.5 %)	14 (38.9 %)	0.89
Hypercholesterolemia ( <i>n</i> (%))	9 (22.5 %)	8 (22.2 %)	0.83
Diabetes ( <i>n</i> (%))	2 (5.0 %)	1 (2.8 %)	0.57
Medication			
Anti-hypertensive drugs ( <i>n</i> (%))	13 (32.5 %)	15 (41.7 %)	0.41
Diuretics ( <i>n</i> (%))	5 (12.5 %)	3 (8.3 %)	0.56
Statins ( <i>n</i> (%))	9 (22.5 %)	5 (13.9 %)	0.33
Other CVD medication ( <i>n</i> (%))	7 (17.5 %)	7 (19.4%)	0.83
Insulin medication ( <i>n</i> (%))	2 (5.0 %)	1 (2.8 %)	0.62

The values, significantly different between groups, are represented in italics

*P* value refers to an unpaired Students *t* test or Pearson's chi-square test between sexagenarians and octogenarians

<sup>a</sup>Ln-transformation was applied as a non-Gaussian distribution was present

circumference divided by hip circumference. Thereafter, resting heart rate and blood pressure were measured twice using an automated sphygmomanometer (M5-1 intellisense, Omron Healthcare, Hoofddorp, the Netherlands) after 5-min supine rest. Finally, all subjects completed a questionnaire concerning their physical activity and health status, in which subjects were asked for medical history, medication usage, daily activity (hours of sport participating per week), and training status (walking-

specific training history in the year preceding the walking march).

#### Thermoregulation

*Intestinal temperature ( $T_{in}$ )* A portable telemetry system (CoreTemp™ system, HQ Inc, Palmetto, USA) was used to measure  $T_{in}$ , which is a safe and reliable method to examine  $T_{in}$  at rest and during exercise (Byrne and Lim 2007; Gant et al. 2006). Subjects ingested an

individually calibrated telemetric temperature pill on the evening preceding the exercise bout, to avoid interaction with fluid ingestion during testing (Wilkinson et al. 2008). Baseline  $T_{in}$  was determined as the average of three consecutive measurements. This procedure was repeated every 5 km along the route. The highest value of these measurements was presented as peak  $T_{in}$  and increment in  $T_{in}$  ( $\Delta T_{in}$ ) was calculated as peak  $T_{in}$  minus baseline  $T_{in}$ .

**Heart rate** Heart rate (HR) was measured simultaneously with  $T_{in}$  (i.e., three consecutive measurements every 5 km) using a 2-channel ECG chest band system (Polar Electro Oy, Kempele, Finland). Mean heart rate during exercise was calculated as the average heart rate, excluding the values measured directly before the start and after the finish. Exercise intensity was calculated by dividing the mean heart rate during exercise by the maximal predicted heart rate ( $208 - 0.7 \cdot \text{age}$ ) (Tanaka et al. 2001).

#### Fluid balance

**Fluid intake** All subjects received written and individual oral instructions regarding the registration of their fluid intake. During exercise, subjects were allowed to drink ad libitum, while they registered the time (in blocks of 1 h), amount (using standard sized cups (125 mL), cans (330 mL) and bottles (500 mL)), and type (“water,” “sports drink,” or “other”) of their individual fluid intake in a diary. Directly post-exercise, the fluid intake diary was checked by the research team, and clarifications were provided by the subjects if necessary. The relative change in body mass (in %) between the measurement immediately before the start and directly after finishing was calculated.

**Sweat rate** Sweat rate (mL/h) was calculated as a combination of body mass, fluid intake, and urine output data using the following formula: sweat rate (mL/h) = (pre-exercise body mass – post exercise body mass + fluid intake – urine output) / exercise duration (Casa et al. 2000).

**Blood sodium levels + plasma volume** Subjects were seated for 5 min after which a 2-mL blood sample was drawn from the antecubital vein. Blood samples were directly analyzed for plasma levels of sodium, hemoglobin, and hematocrit (Rapidpoint® 400, Siemens Healthcare Diagnostics Inc., Tarrytown, New York,

USA). Hyponatremia and hypernatremia were defined as a plasma sodium concentration of  $\leq 135$  and  $\geq 145$  mmol/L, respectively (Adroque and Madias 2000; Hew-Butler et al. 2005). Relative changes in plasma volume were calculated from blood hematocrit and hemoglobin concentrations using Dill and Costill’s equation (Dill and Costill 1974).

**Urine analysis** To determine urine specific gravity, a 5-mL urine sample was provided by all subjects and directly analyzed (Clinitek Status® Analyzer, Siemens Healthcare Diagnostics Inc., Tarrytown, New York, USA). A urine specific gravity of  $\geq 1.020$  g/mL is indicative for dehydration (Casa et al. 2000). To determine the total amount of urine output, subjects were instructed to exclusively urinate into a specialized collecting bag (Roadbag/Ladybag, KETs GmbH, Köln, Germany) during the entire exercise bout. Bags were collected after every 5 km and weighted at the laboratory within 0.1-g accuracy (PT 1400, Sartorius AG, Göttingen, Germany).

**Ambient conditions** During the experiment, dry bulb, wet bulb and globe temperatures were measured every 30 min using a portable climate monitoring device (Davis instruments Inc., Hayward, USA), which is positioned at the start/finish area. The wet bulb globe temperature index (WBGT) was calculated using the formula:  $WBGT = 0.1 (T_{dry\ bulb}) + 0.7 (T_{wet\ bulb}) + 0.2 (T_{globe})$  (Armstrong et al. 2007).

#### Statistical analysis

All values are presented as mean  $\pm$  standard deviation, unless indicated otherwise. Statistical analyses were performed using Statistical Package for Social Sciences 20.0 (IBM SPSS version 20.0, Armonk, New York, USA), and the level of significance was set at  $p < 0.05$ . The Kolmogorov-Smirnov test was used to examine the normality of the data distribution. When data demonstrated a non-Gaussian distribution, Ln transformation was applied. Comparisons of baseline measurements between groups were assessed using an unpaired Student’s *t* test. To assess differences in fluid balance between the sexagenarians and the octogenarians, an unpaired Student’s *t* test was used. A linear mixed model analysis was used to determine whether changes in  $T_{in}$  and exercise intensity across the 30-km exercise differed between both groups. A two-way ( $2 \times 2$ ) repeated

measures ANOVA was used to examine whether exercise altered plasma sodium, hemoglobin, and hematocrit concentration (“exercise”: pre versus post), and whether these changes differed between groups (“group”: sexagenarians versus octogenarians). Finally, a Pearson’s chi-square test was applied to assess differences in the prevalence of dehydration, hypo- or hypernatremia and high urine specific gravities between groups. Results of the Pearson’s chi-square test were presented as relative risk (RR) and their 95 % confidence intervals.

## Results

Three octogenarian subjects did not finish the 30-km walking exercise bout due to a fractured wrist and severe muscle pains and fatigue, and were excluded from further analysis. The average walking distance in the year prior to the march was  $501 \pm 423$  km and  $990 \pm 723$  km for the sexagenarians and octogenarians, respectively ( $p < 0.01$ ). Octogenarians had a lower height, body mass, body mass index (BMI), body fat, lean body mass, and abdominal and waist circumference compared to sexagenarians (all  $p < 0.05$ , Table 1), while no differences were found for mean arterial pressure and heart rate. No differences in prevalence of cardiovascular diseases, hypertension, hypercholesterolemia, and diabetes were reported; additionally, we found no differences in medication usage (Table 1, all  $p > 0.05$ ).

### Exercise characteristics

Relative humidity and wet bulb globe temperature (WBGT) were 95 % and 12 °C at the start of the exercise bout (i.e., 7:00 AM) and gradually changed to 43 % and 24 °C at 5:00 PM. Exercise duration and walking speed were comparable between both groups (Table 2). Exercise intensity increased during the exercise bout to a similar extent in both groups ( $p = 0.88$ , Fig. 1a).

### Intestinal temperature

Baseline intestinal temperature ( $T_{in}$ ) was 0.3 °C lower in the octogenarians ( $37.2 \pm 0.3$ ) compared to the sexagenarians ( $37.5 \pm 0.4$ ,  $p < 0.01$ ).  $T_{in}$  increased significantly during exercise ( $p < 0.01$ ), while the increase in  $T_{in}$  was significantly larger in octogenarians compared to sexagenarians ( $1.2 \pm 0.5$  °C versus  $0.7 \pm 0.4$  °C,  $p < 0.01$ ). In

contrary, maximum  $T_{in}$  tended to be higher in the octogenarians ( $38.4 \pm 0.4$  °C versus  $38.2 \pm 0.3$  °C;  $p = 0.09$ ).

### Fluid balance

Average fluid intake during exercise was lower for the octogenarians ( $251 \pm 97$  mL/h) compared to the sexagenarians ( $325 \pm 125$  mL/h,  $p = 0.01$ ) (Fig. 2a). Furthermore, octogenarian subjects demonstrated a significantly lower urine output compared to sexagenarians ( $p < 0.01$ ), and the sweat rate tended to be lower in the octogenarian group ( $p = 0.07$ ) (Fig. 2b, c). Post-exercise plasma volume, plasma sodium, hemoglobin and hematocrit concentration, and incidence of urine specific gravity levels  $\geq 1.020$  g/mL were not different between groups (all  $p > 0.05$ , Table 2). Both groups demonstrated an exercise-induced increase in hemoglobin and hematocrit concentration ( $p < 0.01$ ), while the exercise-induced changes in plasma sodium concentration were not different in both groups ( $p > 0.05$ ). Moreover, both groups demonstrated a decrease in body mass after exercise. The relative decrease in body mass was significantly larger for the octogenarians ( $p = 0.04$ , Fig. 2d), while no differences between groups were observed in dehydration status (Table 2).

## Discussion

The purpose of our study was to compare thermoregulatory responses and fluid balance during prolonged, moderate-intensity exercise between sexagenarians and octogenarians. We found that octogenarians demonstrate a lower baseline  $T_{in}$  and a larger rise in  $T_{in}$  during prolonged walking exercise compared to sexagenarians. As workload and exercise intensity were similar between groups, these results may suggest that the thermoregulatory control declines with advanced age. Moreover, octogenarians reported a lower fluid intake and urine output in combination with a larger loss of body mass compared to sexagenarians, while other fluid balance parameters were not different between groups. Thus, in moderate ambient conditions and with ad libitum fluid intake, the fluid balance regulation of both groups was adequate to avoid development of sodium disturbances or severe dehydration.

In accordance with literature, baseline  $T_{in}$  was 0.3 °C lower in octogenarians compared to sexagenarians (Howell 1948). The most likely explanations for this

**Table 2** Exercise characteristics and fluid balance presented per age group

Parameter	Sexagenarians	Octogenarians	<i>P</i> value
Exercise characteristics			
Exercise duration (hh:mm)	7:24±0:58	7:45±0:57	0.13
Average walking speed (km/h) <sup>a</sup>	4.1±0.5	3.9±0.5	0.13
Average HR (bpm)	116±15	106±16	<0.01
Average exercise intensity (% of HR max)	70±9	70±11	0.97
Baseline $T_{in}$ (°C)	37.5±0.4	37.2±0.3	<0.01
$\Delta T_{in}$ (°C)	0.7±0.4	1.2±0.5	<0.01
Peak $T_{in}$ (°C)	38.2±0.3	38.4±0.4	0.09
Fluid balance parameters			
Fluid intake (mL/h)	325±125	251±97	0.01
Water (%)	62.3±26.1	51.7±30.9	0.11
Other (%)	37.7±26.1	48.3±30.9	0.11
Sweat rate (mL/h)	364±148	294±150	0.07
Urine output (mL/h) <sup>a</sup>	52±40	28±22	<0.01
Urine specific gravity $\geq 1.020$ g/mL (n(%))	33 (80.5 %)	31 (75.6 %)	0.88
Sodium concentration (mmol/L) <sup>a</sup>	142.0±3.3	141.1±4.5	0.33
Prevalence of hyponatremia (n(%))	1 (2.5 %)	3 (7.7 %)	0.24
Prevalence of hypernatremia (n(%))	6 (15.0 %)	5 (12.8 %)	0.94
Hemoglobin (mmol/L)	9.7±0.8	9.4±0.8	0.15
Hematocrit (L/L) <sup>a</sup>	45.8±3.8	44.7±3.7	0.22
Plasma volume change (%) <sup>a</sup>	-3.9±5.3	-3.5±6.2	0.19
Body mass change (kg)	-0.7±0.8	-0.8±0.7	0.36
Body mass change (%)	-0.7±0.9	-1.2±1.0	0.04
Prevalence of dehydration ( $\geq 2$ % body mass loss)	2 (5.0 %)	6 (15.4 %)	0.10

The values, significantly different between groups, are represented in italics

$T_{in}$  intestinal temperature, *HR* heart rate

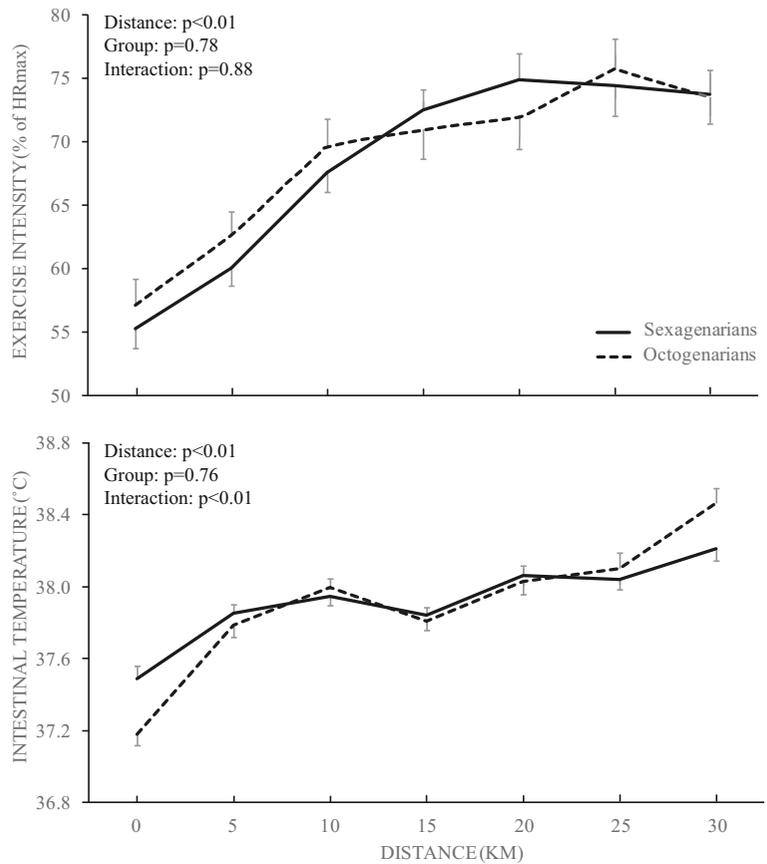
*P* value refers to an unpaired Students *t* test or Pearson's chi-square test between sexagenarians and octogenarians

<sup>a</sup>Ln-transformation was applied as a non-Gaussian distribution was present

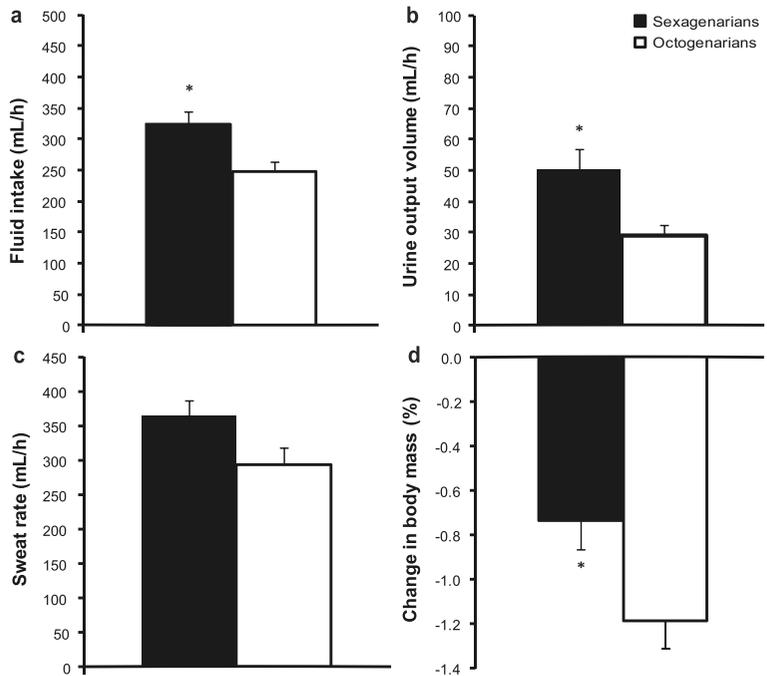
observation is the smaller muscle mass and, concomitant, lower metabolic heat production in the oldest group (Blatteis 2012; Howell 1948). Furthermore, higher age was associated with a significantly higher exercise-induced increase in  $T_{in}$ . Since exercise characteristics were similar between groups (i.e., duration and intensity), we propose three possible explanations for the higher increase in  $T_{in}$  in the octogenarians. First, the sweat rate tended to be lower in aged subjects due to a decrease in individual sweat gland output as well as a reduced number of activated sweat glands by heat (Blatteis 2012; Shibasaki and Crandall 2010). Presumably, when aging progresses, the sweat rate response is further impaired, which may result in a larger increment of  $T_{in}$  during prolonged exercise. Second, the initiation

of the thermoregulatory responses to heat exposure occurs by altered signaling from thermosensors located both in the periphery and the core of the body (Blatteis 2012). With advanced age, there is a higher peripheral fiber loss and a lower conduction velocity of the nervous system, which may delay vascular responses to heat. This results in a decreased peripheral thermal sensation, a delayed response to heat exposure (Blatteis 2012; Guergova and Dufour 2011), and subsequently a larger exercise-induced increase in  $T_{in}$ . A third possible mechanism relates to the attenuated cutaneous vasodilator response to heat in aged people (Holowatz et al. 2010, 2007). Both, a delay in the initiation of the vasodilatory as well as a decreased vasodilatory capacity of the skin are known to limit heat dissipation (Holowatz et al.

**Fig. 1** Time course of **a** exercise intensity (% of HRmax) and **b** intestinal temperature (°C) in sexagenarians (*solid line*) and octogenarians (*dashed line*). While octogenarians demonstrated a larger increase in intestinal temperature compared to sexagenarians ( $p < 0.01$ ), no differences were observed in the change in exercise intensity between the groups ( $p = 0.88$ ). Data is presented as mean  $\pm$  SEM



**Fig. 2** Fluid balance parameters (**a** fluid intake, **b** urine output, **c** sweat rate, and **d** body mass loss) presented for both sexagenarians (*black bars*) and octogenarians (*white bars*). Sexagenarians demonstrated a higher fluid intake and sweat rate and subsequently a lower body mass loss. Data were presented as mean  $\pm$  SEM. \* represents a significant difference between both groups



2010, 2007). The above-mentioned factors limit the human skin to lose heat, thereby causing  $T_{in}$  to rise and potentially increase the risk to develop heat-related illnesses. In parallel, a trend ( $p=0.09$ ) for differences in maximum  $T_{in}$  between octogenarians and sexagenarians was observed. While the present study was performed in moderate ambient conditions and maximum  $T_{in}$  of both groups was within the normal physiological range, differences in maximum  $T_{in}$  may be more pronounced under hot and humid ambient conditions. In summary, our data may suggest a progressive age-related impairment of thermoregulatory responses, with larger exercise-induced  $T_{in}$  increases in octogenarians compared to sexagenarians.

Octogenarians demonstrated a larger relative body mass loss compared to sexagenarians, suggesting a deficiency in fluid intake and/or a higher fluid loss. Indeed, the self-reported fluid balance diaries demonstrated that octogenarians consumed 74 mL/h less fluid compared to sexagenarians. This observation is in line with Phillips et al. (Phillips et al. 1984) who demonstrated that older subjects (67–75 years) reported a lower thirst sensitivity compared to young controls (20–31 years) after a 24-h period of water deprivation. The blunted thirst response in older humans was later confirmed by others, who compared young and old subjects (Kenney and Chiu 2001; Mentis 2006). Next to the differences in fluid intake, the octogenarians also reported a lower urine output. It may be assumed that there is a causal relationship between the lower fluid intake and the lower urine output. The lower total body water content may enhance water reabsorption in the kidneys and hence reduce the urine production. Despite the lower fluid intake and urine output, no differences were observed between groups for changes in body mass, blood sodium concentration, and plasma volume. These findings suggest that octogenarians and sexagenarians are both capable to regulate their fluid balance appropriately during prolonged walking exercise.

*Clinical relevance* Health problems like heat stroke, dehydration, and hyponatremia are frequently reported during prolonged exercise. Although octogenarians reported a larger  $T_{in}$  increase and a lower fluid intake compared to sexagenarians, medical problems did not occur in both groups. Risk assessment must be revised when these vulnerable groups perform exercise in hot and humid ambient conditions. Particularly, since recent fluid intake guidelines advice athletes to drink according

to their thirst sensation to avoid dehydration (Dion et al. 2013; Goulet 2011) and previous studies noted a decreased thirst sensitivity in elderly (Kenney and Chiu 2001; Mack et al. 1994; Phillips et al. 1984), for that reason, the “drink according to thirst” regimen may be inappropriate in the older individuals under severe ambient conditions. Therefore, it is recommended that future fluid replacement guidelines should include specific information on rehydration in elderly.

*Limitations* This study includes two unique groups of advanced aged subjects that performed prolonged walking exercise. However, one might argue that these individuals do not represent the general advanced age population, since these subjects have a highly active lifestyle. Consequently, there may be selection bias in the current study. Fact is that medical advances stimulate healthy aging and subsequent longevity in modern society. As a direct consequence, physical activity is increasingly popular in this population, which is reinforced by the growing number of older humans that participate in endurance exercise events. Therefore, insight into the physiological responses of these individuals is necessary for a safe exercise environment with appropriate recommendations. Secondly, sexagenarians reported a significantly higher BMI and lean body mass compared to octogenarians. As these characteristics increase the risk for fluid disturbances and the development of dehydration in sexagenarians (Eijsvogels et al. 2011), we may have underestimated the differences in thermoregulatory and fluid balance responses between both groups. A third limitation of this study is the used estimation of the maximum heart rate to calculate the exercise intensity. Since we used the formula by Tanaka et al. (Tanaka et al. 2001), a widely used method to calculate the predicted maximum heart rate, we believe that the consecutively calculated exercise intensities properly reflect the real exercise intensities. Finally, clothing was not reported during the study, while it plays an important role in the capacity to dissipate heat. However, the majority of the subjects wore normal walking clothes (t-shirt+shorts), and therefore, we strongly believe that clothing did not influence our results.

In conclusion, octogenarians demonstrate an impaired thermoregulatory control compared to sexagenarians during prolonged moderate-intensity exercise under moderate ambient conditions. In contrast, fluid balance

was well controlled in both groups and did not deteriorate with aging. This enabled sexagenarians as well as the octogenarians to successfully complete a 30-km walking march without heat- or fluid-related health problems.

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**Conflict of interest** None.

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