Heat Acclimation and Physical Training Adaptations of Young Women

Using Different Contraceptive Hormones

Manuscript No. E-00434-2004

Lawrence E. Armstrong1,2, Carl M. Maresh1,2, NiCole R. Keith1, Tabatha A. Elliott1, Jaci L. VanHeest1, Timothy P. Scheett1, James Stoppani1, Daniel A. Judelson1, Mary Jane De Souza1,3

1 Human Performance Laboratory, Department of Kinesiology, University of Connecticut, Storrs CT 06269
2 Department of Physiology and Neurobiology, University of Connecticut, Storrs, CT 06269
3 Women’s Exercise and Bone Health Laboratory, Department of Exercise Science, University of Toronto, Toronto, ON, Canada M5S 2W6

RUNNING HEAD: Heat acclimation and training: contraceptive effects

CONTACT INFORMATION: Lawrence E. Armstrong, Ph.D., FACSM
Human Performance Laboratory
Unit 1110, 2095 Hillside Road
Storrs, CT 06269-1110
Office: (860) 486-2647
FAX: (860) 486-1123
e-mail: lawrence.armstrong@uconn.edu

Copyright © 2004 by the American Physiological Society.
Abstract

Although endogenous and exogenous steroid hormones affect numerous physiologic processes, the interactions of reproductive hormones, chronic exercise training and heat acclimation are unknown. This investigation evaluated the responses and adaptations of 36 inactive females (mean ± SD; age, 21 ± 3y) as they undertook a 7-8 wk program (HAPT) of indoor heat acclimation (90 min·d⁻¹, 3 d·wk⁻¹) and outdoor physical training (3 d·wk⁻¹) while using either an oral estradiol-progestin contraceptive (ORAL, n=15), a contraceptive injection of depot medroxyprogesterone acetate (DEPO, n=7), or no contraceptive (EU-OV, n=14; control). Standardized physical fitness and exercise-heat tolerance tests (EHT; 36.5°C, 37%rh), administered before and after HAPT, demonstrated that the three subject groups successfully (P<0.05) acclimated to heat (i.e., rectal temperature, heart rate) and improved muscular endurance (i.e., situps, pushups, 4.6-km run time), and body composition characteristics. The stress of HAPT did not disrupt the menstrual cycle length/phase characteristics, ovulation, or plasma hormone concentrations of EU-OV. No between-group differences (P>0.05) existed for rectal and skin temperatures, metabolic, cardiorespiratory, muscular endurance, or body composition variables. A significant difference post-HAPT in the onset temperature of local sweating, ORAL (37.2 ± 0.4°C) versus DEPO (37.7 ± 0.2°C), suggested that steroid hormones influenced this adaptation.

In summary, virtually all adaptations of ORAL and DEPO were similar to EU-OV, suggesting that exogenous reproductive hormones neither enhanced nor impaired the ability of women to complete 7- 8 wk of strenuous physical training and heat acclimation.

Key words: estrogen, progesterone, estradiol, progestin, rectal temperature
Introduction

During the past two decades, animal and human investigations have clarified the neuroendocrine interactions between the thermoregulatory and reproductive systems. Specifically in mature rats, estradiol (E₂) applied to in vitro tissue slices increased the activity and firing rate of warm-sensitive neurons but not cold-sensitive neurons, harvested from the preoptic area of the anterior hypothalamus (32), whereas progesterone (P₄) decreased these responses in warm-sensitive neurons and increased them in cold-sensitive neurons (28). In humans, it is generally recognized that E₂ decreases body temperature by enhancing effector responses (i.e., sudomotor and vasomotor) and heat loss (31).

Conversely, systemic P₄ increases core temperature (6), suggesting an upward shift in the thermoregulatory set-point (24). These observations agree with the known changes of E₂, P₄, and body temperature during the human menstrual cycle. During the early follicular phase, both serum E₂ and P₄ concentrations are low; during the late follicular phase, E₂ is elevated and P₄ is low; and both E₂ and P₄ are high during the mid-luteal phase (12, 23). Due to these hormone fluctuations, body temperature during the luteal phase is approximately 0.3 to 0.5°C higher than during the late follicular phase (17, 23, 34).

Several human studies have utilized oral exogenous hormones to evaluate the effects of ethinyl estradiol (EE) and progestins on exercise performance (9, 26), thermoregulation in mild (24 – 28 °C) (14, 21, 31) and hot (30 – 36 °C) (34-36) one reference inserted here environments, exercise responses while wearing impermeable suits at 40°C (37), and resting vasomotor responses while wearing a water-perfused suit heated to 38.5°C (15). However, little is known about the interactions of exogenous hormones, chronic exercise training and heat acclimation. Therefore, the purpose of the present investigation was to measure the thermal, metabolic, cardiorespiratory,
performance, body composition, and perceptual responses and adaptations of healthy young women, as they participated in a 7 – 8 wk program (i.e., spanning two menstrual cycles) of heat acclimation and physical training (HAPT). Each woman was voluntarily using either oral contraceptive pills, an injectable steroid contraceptive, or no contraceptive (control).

This research is important and relevant to the unique physiological adaptations and lifestyles of women, in two ways. Firstly, approximately 11.2 million women in the United States, aged 18 – 44 y, utilize a pharmacologic contraceptive preparation (13), 28% of all women engage in regular leisure-time physical activities (5), and outdoor exercise is more common during summer months when ambient temperatures are high. The effects of programs like HAPT on reproductive physiology are unknown. Because the present investigation involved moderate-to-high intensity exercise, we hypothesized that HAPT would not alter menstrual cycle length/phase characteristics, ovulation, or plasma hormone concentrations in the eumenorrheic-ovulatory (control) subjects. Secondly, because the steroid environments of synthetic steroid preparations and the ovarian steroid hormones of the menstrual cycle are markedly different, it is possible that oral estrogen-progestin formulations and injectable depot medroxyprogesterone acetate (MPA) influence heat acclimation and physical training adaptations differently. However, after considering that three days of estrogen supplementation had no effect (i.e., in adult women using no contraception) on heat transfer to the skin or evaporative cooling during exercise (14), we hypothesized that physiological responses and adaptations would be similar in the three experimental groups throughout HAPT.

Methods
**Subjects.** Thirty-six healthy females (mean ± SD; age, 21 ± 3 y; maximal aerobic power \(\text{VO}_2\text{max}\), 37.1 ± 4.1 ml·kg\(^{-1}\)·min\(^{-1}\); height, 162.5 ± 7.6 cm; body mass, 65.78 ± 11.11 kg) were selected as subjects, from a sample of 72 potential participants. Fourteen women were eumenorrheic, had documented normal ovulatory patterns, were not taking exogenous contraceptive preparations, and served as the control group (EU-OV). Fifteen other participants used oral EE-progestin contraceptives (ORAL), and seven received contraceptive injections of depot MPA (DEPO) (Depo-Provera™, Pfizer Incorporated, New York, NY). Subjects did not change their pre-study contraceptive status (i.e., previous three months) during this investigation. The University of Connecticut Institutional Review Board for Human Studies approved all procedures. During the month of October, potential subjects attended an informational briefing, completed medical history questionnaires, and provided their signed informed consent to participate.

**Preliminary Screening.** During the month of November, exercise history and eating disorder questionnaires were completed and evaluated. Likely candidates for each treatment group then provided an initial venous blood sample. This sample was analyzed to verify self-reported non-pregnancy and normal thyroid function (i.e., prolactin [PRL], thyroid-stimulating hormone, free thyroxin \([T\text{_{4}}]\)). Each participant provided documentation of a recent medical examination, permission of her personal physician to participate in this research, and a normal Papanicolaou smear. Other exclusionary criteria included: change in menstrual pattern or contraceptive use within the previous three months, use of oral contraceptive preparations containing mestranol, recent heat exposure or heat acclimatization, previous history of exertional heat illness, major depression, acute or chronic illness (i.e., of a respiratory, cardiovascular, convulsive, hypertensive, or metabolic
nature), self-reported frequent physical training, tobacco use, and drug or alcohol
dependence.

**Secondary Screening.** Normal ovulatory menstrual physiology was evaluated for
EU-OV subjects via venous fasting blood sample analyses (E₂, P₄, luteinizing hormone [LH],
follicle-stimulating hormone [FSH], and sex hormone binding globulin [SHBG]), during
November and December, in three phases. First, subjects reported to the laboratory for
venipuncture within 2–5 days of the first day of menses to verify the early follicular phase
via E₂ and P₄ serum analyses. Samples were then collected every third day until
approximately days 9–11 (dependent on previous menstrual cycle length), and then daily
to document the occurrence of an LH surge. In these samples, E₂, P₄, and LH were
assayed. After the documentation of an LH surge, blood samples were collected every third
day until the end of the cycle, to document luteal phase characteristics. Subjects who failed
to demonstrate an LH surge or who had a luteal phase length less than 10 days and a mid-
luteal P₄ less than 15.9 nmol·L⁻¹, were excluded from the study. These analyses were
continued for the duration of the study. Analyses of circulating reproductive hormones were
performed at the Department of Fertility and Reproductive Endocrinology, New Britain
General Hospital, New Britain, CT.

All subjects voluntarily ingesting oral contraceptives (ORAL group) reported the
exact preparation, as shown in Table 1, and duration of use. All oral preparations contained
EE and a second or third generation progestin. These subjects also provided investigators
with empty pill packs, at the end of each month, to document therapy details. Compliance
to therapy was evaluated by measuring the concentration of plasma EE, prior to training
and at the end of training.

[INSERT TABLE 1 HERE]
All subjects voluntarily receiving long-acting, injectable depot MPA contraceptive therapy (DEPO group) reported the exact dose, duration of use, and compliance to therapy. The concentration of plasma MPA was measured in serum, prior to training and at the end of training, to verify compliance.

Serum reproductive hormones and PRL were analyzed using a chemiluminescence based immunoassay analyzer (Immulite®, Diagnostic Products Corporation, Los Angeles, CA). The analytical sensitivity of the E2 assay was 55 pmol\cdot L^{-1}. The intra-assay and inter-assay coefficients of variation were 8.6% and 9.5%, respectively. The analytical sensitivity of the P4 assay was 0.3 nmol\cdot L^{-1}. The intra-assay and inter-assay coefficients of variation were 5.9% and 5.6%, respectively. The analytical sensitivity of the LH assay was 0.1 IU\cdot L^{-1}. The intra-assay and inter-assay coefficients of variation were 10.6% and 8.1%, respectively. The analytical sensitivity of the SHBG assay was 0.2 nmol\cdot L^{-1}. The intra-assay and inter-assay coefficients of variation were 9.3% and 10.4%, respectively. The analytical sensitivity of the PRL assay was 0.5 \mu g\cdot L^{-1}. The intra-assay and inter-assay coefficients of variation were 6.3% and 6.8%, respectively.

**Standardization of Testing.** Blood hormone analyses were used to determine the first day of heat acclimation and physical training (HAPT) for EU-OV subjects, such that exercise sessions began during the first week of the follicular phases. All ORAL subjects took pills that contained both oral contraceptives (days 1 – 21) and placebo pills (days 22-28). The starting date of HAPT for ORAL was defined as day 1 of the 28-day pill pack, when they began ingesting the estrogen-progestin contraceptive. Thus, the hormone levels for both EU-OV and ORAL were similar (i.e., low) at the beginning of HAPT. The starting date for DEPO was defined as day 1 of an arbitrary 28-day period, as assigned by investigators.
Subjects performed the standardized exercise-heat tolerance test (EHT), before and after HAPT, as follows: EU-OV, on days 2 – 5 of the menstrual cycle; ORAL, on days 2 – 5 of the placebo phase of the pill pack; DEPO, on days 2 – 5 of the arbitrary 28-day period. Twenty-four to 48 h prior to both EHT, measurements of sweat sensitivity, body composition and VO_{2max} were conducted. These features of the experimental design ensured that the pre-HAPT and post-HAPT measurements were conducted at the same point relative to reproductive hormones.

**Body Composition and VO_{2max}**. Each subject was weighed under water, while suspended from a computer-monitored strain gauge, and on land, to determine body density as described elsewhere (1). From body density values, the percent body fat and the fat-free mass were calculated according to published normative equations (33). On the same day, each subject completed an incremental run on a motorized treadmill to determine VO_{2max}. This test began at a brisk pace (i.e., based on each individual’s previous running experience or estimated time to complete a 5 km run; approximately 75% VO_{2max}) for four minutes at 0% grade. The treadmill grade was then increased to 4% for two minutes. At two minute intervals thereafter, the grade was further increased 2% until the subject reached volitional exhaustion. Oxygen uptake, minute ventilation and respiratory exchange ratio were measured every 30 sec, via indirect calorimetry (metabolic cart, model CPX/D, Medical Graphics Corporation, St. Paul, MN). Gas concentrations and flow volume were calibrated to manufacturer specifications prior to each VO_{2max} test, using certified O_{2}-CO_{2} gas mixtures and a 3-liter calibration syringe (model R5530, Vacumed, Ventura, CA).

Two of the following criteria verified the attainment of VO_{2max}: an increase of oxygen consumption less than 150 ml·min⁻¹ despite an increase in work load, or heart rate (Vantage XL heart rate monitor, Polar Electro Inc., Woodbury, NY) greater than 90% of predicted maximum (220 beats·min⁻¹ minus chronologic age), or respiratory exchange ratio (V_{CO2}/V_{O2})
Both the body composition and the VO$_{2\text{max}}$ procedures were repeated at the end of training.

*Heat Acclimation/Physical Training Program.* After successfully completing screening procedures, subjects participated in 7 – 8 wk (i.e., two menstrual cycles) of supervised heat acclimation and physical training (HAPT). HAPT occurred on six days per week, as three outdoor sessions and three indoor sessions, during the months of January and February. Outdoor physical training and indoor heat acclimation activities, in both cold and hot environments, were combined to simulate the variety of stressors encountered by athletes and military personnel.

Outdoor physical training involved strenuous running for 4.6 km at an individualized pace (i.e., exercise intensity estimated at 60 – 85%VO$_{2\text{max}}$), pushups, and situps; exercise intensity increased weekly as training progressed. During morning running sessions, the mean (+ SD) outdoor air dry bulb and dew point temperatures were -3.9 ± 0.8 and -5.9 ± 0.9°C, respectively. Adaptations to the 7 – 8 wk physical training program were evaluated as change in body composition and fitness test scores (before versus after HAPT). The physical fitness tests measured the maximum number of standardized situps and pushups that could be completed in 60 sec, as well as a timed 4.6 km competitive footrace.

Indoor 90 min heat acclimation sessions took place in climatically-controlled conditions of 36.0 – 37.0°C and 33 – 37% relative humidity (%rh); chilled drinking water was consumed *ad libitum*. Partial results concerning water consumption and thirst have been reported elsewhere (29). Exercise-heat exposure employed a circuit of bench stepping, stair climbing, cycle ergometry, and walking on a motorized treadmill at approximately 50 – 70 %VO$_{2\text{max}}$ for each mode. Subjects were encouraged to exercise continuously for 90 min, and after the initial 1 - 3 days subjects accomplished 90 min per day with very few, sporadic
exceptions. If a subject voluntarily stopped exercising because of fatigue, she rested in the heat for the entire 90-min period. Subjects inserted a rectal thermistor (YSI 401 rectal probe, Yellow Springs Inc., Yellow Springs, OH) 10 cm beyond the external anal sphincter and wore a heart rate monitor, to allow rectal temperature ($T_{re}$) and heart rate (HR), respectively, to be monitored. If a subject announced that she was unable to continue exercising, she was encouraged to rest in the environmental chamber for the entire 90 min session. However, subjects were removed from the environmental chamber if one of the following predetermined criteria was reached: $T_{re} \geq 39.5 ^\circ C$, HR > 180 beats·min$^{-1}$ for five consecutive minutes, or signs of exertional heat illness. A trained investigator monitored all training sessions.

**Exercise-Heat Tolerance Testing.** Subjects performed a standardized exercise-heat tolerance test (EHT) before and after HAPT; both EHT began at the same point relative to the reproductive hormone cycle and at the same time within-day. Water deprivation (24 h) plus mixed aerobic activity (2 – 3 h) were utilized to induce dehydration, prior to each EHT. This dehydration was incorporated to increase the total stress experienced by test subjects. Body mass ($\pm 50 \text{ g}$; floor scale, model DS44L, Ohaus, Pine Brook, NJ) was measured immediately before and after water restriction plus activity, to ensure the desired 3.0% body mass loss; also, if urine specific gravity (refractometer) did not exceed 1.020, the test was not conducted. Body mass again was measured upon entering, and immediately before leaving, the environmental chamber. These hydration procedures were controlled during both EHT, before and after HAPT.

Once inside the chamber ($36.5 \pm 1.4 ^\circ C$, $37 \pm 1\%$ rh, $2.3 \text{ m·s}^{-1}$ air flow), subjects stood quietly for 20 minutes, to allow body fluids, plasma volume and skin temperatures to stabilize. After this equilibration, subjects walked on a motorized treadmill at 93.6 m·min$^{-1}$
and 5% grade. Treadmill belt speed was verified during each test with a hand-held
tachometer (Model 8240-20, Cole Parmer Instrument Company, Chicago, IL). No water
was consumed during the EHT. Physiological criteria for test termination were identical to
those used during indoor heat acclimation sessions (above). In addition, EHT were stopped
if subjects verbally announced volitional exhaustion or completed the entire 90 min of
exercise.

Throughout the EHT, physiological and perceptual measures were obtained, as
depicted in Figure 1. Changes in body mass were used to calculate whole body sweat rate
(SRwb), corrected for urine production and sweat absorbed by clothing. Blood pressure was
measured with a sphygmomanometer and stethoscope, immediately prior to exercise and at
30 min intervals thereafter. HR (Vantage XL heart rate monitor, Polar Electro Inc.,
Woodbury, NY), Tr (YSI 401 Rectal Probe, Yellow Springs Incorporated, Yellow Springs,
OH), and ratings of perceived exertion (6 - 20 point scale; (8)) were determined at rest,
every fifteen minutes during, and at the conclusion of the EHT. Measures of oxygen uptake
were obtained at 20 and 40 min of exercise using the metabolic cart described above. Total
exercise time was recorded to the nearest second.

[INSERT FIGURE 1 HERE]

Skin temperatures were measured at three sites, at the end of the EHT. During the
standing equilibration, approximately 6 cm² of skin over the left lateral mid-calf, the left
dorsal mid-forearm, and the left pectoralis muscle (at the mid-point between the subject’s
nipple and mid-clavicle) were shaved and cleaned with alcohol. Skin temperatures were
measured at these sites with an infrared temperature scanner (Ototemp Model HTTS-3000,
Exergen Corporation, Watertown, MA). Mean weighted skin temperature (Tsk) was
calculated using this formula (11): Tsk = 0.5 (chest) + 0.14 (forearm) + 0.36 (calf).
Sweat Sensitivity. Within 24 h of both EHT, a separate experiment was administered to determine sweat sensitivity. Upon arrival at the laboratory, euhydration was verified by a urine specific gravity measurement < 1.020. Subjects inserted a rectal thermistor (YSI 401 Rectal Probe, Yellow Springs Incorporated, Yellow Springs, OH) 10 cm beyond the external anal sphincter. Exercise involved approximately 20 min of cycle ergometry at 45% VO₂max while in a euhydrated state, in a mild environment (22.5°C). This test was designed to eliminate dehydration and high ambient temperature as confounding factors. Local sweat rate was measured continuously with a dew point sensor (model BI-2, Bi-Tronics Corp., Guilford, CT) attached with surgical cement to the skin above the medial border of the left scapula. Tₑ was observed with a digital thermometer (± 0.1 °C) concurrent with each local sweat rate measurement. The threshold temperature for the onset of sweating was identified by observing the inflection point (i.e., from resting baseline sweat rate) during continuous measurements. Sweat sensitivity was defined as the slope of the relationship between rectal temperature plotted against local sweat rate (i.e., statistical regression of the linear segment).

Blood analyses. Immediately prior to, and at the conclusion of the EHT, blood samples were obtained via indwelling cannula (JELCO4054, Johnson and Johnson Medical Incorporated, Arlington, TX). Posture was controlled (see above). Ten mL of blood were withdrawn into a plain glass tube (Becton, Dickinson and Company, Franklin Lakes, NJ) for the measurement of hematocrit and hemoglobin. Hematocrit was determined in triplicate via the microcapillary technique. Hemoglobin was measured in triplicate via the cyanmethemoglobin method (Kit 525, Sigma Chemical Incorporated, St. Louis, MO). Percent change in plasma volume was calculated using hematocrit and hemoglobin values (19). An additional 10 mL of blood was withdrawn into a chilled glass tube (Becton, Dickinson and
Company, Franklin Lakes, NJ) pre-treated with EDTA for the measurement of plasma glucose and lactate. These samples were centrifuged at 3000 rpm and 4.0°C for 15 min. Plasma lactate and glucose values were determined in duplicate using a glucose and L-lactate analyzer (model 2300, Yellow Springs Instruments, Yellow Springs, OH).

*Data analyses.* The mean and standard deviation were calculated for all variables. Variables were evaluated with repeated measures, univariate or multivariate analysis of variance to calculate differences between groups, across time, and/or their interaction where appropriate. Significant F-ratios were further evaluated with Tukey’s *post hoc* test. Significance was established at $P < 0.05$.

**Results**

*Reproductive hormones.* The concentrations of $E_2$, $P_4$ and PRL in all groups (P>0.05) did not change during the course of HAPT (not acclimated [NA] versus heat-acclimated [ACCL] values, Table 2). Significantly higher levels of SHBG ($P < 0.05; \text{before versus after HAPT}$) were measured in ORAL (versus EU-OV and DEPO). Although HAPT enhanced physical fitness and induced heat acclimation in all groups (see below), no main effects (between-group or across time; $P > 0.05$) were observed for $E_2$ and $P_4$ concentrations. However, PRL significantly increased during HAPT ($P < 0.05$) in EU-OV (+33.3%), but not ORAL (+3.4%) or DEPO (+24.3%).

All menstrual cycles of EU-OV, before and after the HAPT intervention, were ovulatory. In EU-OV, menstrual cycle length (before HAPT, $27.1 \pm 0.6 \text{ d}$; after HAPT, $28.1 \pm 1.0 \text{ d}$), day of ovulation (before HAPT, $15.2 \pm 0.4 \text{ d}$; after HAPT, $15.2 \pm 0.6 \text{ d}$), follicular phase length (before HAPT, $15.2 \pm 0.4 \text{ d}$; after HAPT, $15.2 \pm 0.6 \text{ d}$), and luteal phase length (before HAPT, $11.9 \pm 0.3 \text{ d}$; after HAPT, $12.8 \pm 0.4 \text{ d}$) were not altered by HAPT.
The menstrual cycle characteristics, as described by hormone concentrations, are presented in Table 2. Concentrations of E\textsubscript{2} measured mid-cycle (before HAPT, 852.0 ± 81.5 pmol·L\textsuperscript{-1}; after HAPT, 915.9 ± 91.4 pmol·L\textsuperscript{-1}) and mid-luteal phase (before HAPT, 483.1 ± 57.6 pmol·L\textsuperscript{-1}; after HAPT, 487.9 ± 40.4 pmol·L\textsuperscript{-1}) were unaffected by HAPT. Similarly, mid-luteal measurements of P\textsubscript{4} (before HAPT, 12.9 ± 1.4 ng·ml\textsuperscript{-1}; after HAPT, 12.5 ± 0.9 mg·dl\textsuperscript{-1}) and peak LH concentration (before HAPT, 44.4 ± 4.1 IU·L\textsuperscript{-1}; after HAPT, 44.1 ± 4.9 IU·L\textsuperscript{-1}) were unchanged by HAPT. The entire HAPT program was completed with high compliance (95% participation) for daily outdoor and indoor sessions.

[INSERT TABLE 2 HERE]

**Physical Training.** Table 3 presents the measurements that indicated improved physical fitness in all groups. The following variables significantly (P <0.05) improved in all groups, indicating improved physical fitness following the 7 – 8 wk of HAPT intervention. Pushups per 60 sec (+134.2%), situps per 60 sec (+46.3%), 4.6 km run time (-24.7%), maximal aerobic power (VO\textsubscript{2max}, +9.4%), body fat percentage (-6.7%), and fat-free mass (+3.0%) were all significantly improved across the groups. Due to an increase in fat free mass and a decrease in fat mass, body mass did not change significantly during HAPT (+0.3%, P > 0.05). No between-group differences existed for any variable. All body composition variables were similar for ORAL, EU-OV and DEPO throughout HAPT (NA versus ACCL values).

[INSERT TABLE 3 HERE]

**Heat Acclimation.** Pre-exercise dehydration level, prior to EHT, was statistically similar across time and between groups (initial EHT, -2.8 ± 0.6%; final EHT, -2.9 ± 0.6% body weight; all groups combined). E\textsubscript{2} and P\textsubscript{4} were similar in all groups, on the morning of EHT tests (Table 2).
The influence of HAPT on resting $T_{re}$ and $T_{sk}$ was evaluated. A significant ($P < 0.03$, $n = 34$) main effect of time, with no between-group difference, was detected for pre-exercise $T_{re}$. The mean ($\pm$ SD) baseline $T_{re}$ values of all groups combined, measured immediately prior to entering the environmental chamber, were $37.2 \pm 0.3^\circ C$ (before HAPT) and $37.1 \pm 0.3^\circ C$ (after 7 – 8 wk of HAPT). The baseline $T_{sk}$ values, measured at the same time as $T_{re}$, were not influenced by HAPT (before, $32.2 \pm 0.8^\circ C$; after, $32.2 \pm 0.9^\circ C$).

The following variables indicated that all groups achieved heat acclimation. Total exercise time increased after HAPT by 70.9% ($P < 0.05$), but no mean differences were detected between treatment groups. Final $SR_{wb}$ was not affected by HAPT (Table 4) or contraceptive type. When considered at identical time points in both EHT (i.e. the time at which the initial EHT ended), 7 – 8 wk of HAPT significantly decreased final HR, final $T_{re}$, final $T_{sk}$, and final rating of perceived exertion, regardless of group. Oxygen consumption during the EHT decreased by 4.4% (-0.9 ml·kg$^{-1}·$min$^{-1}$; initial EHT versus final EHT), when measured at identical treadmill speeds, despite similar body masses. As noted above, $VO_{2\text{max}}$ significantly increased during this investigation. Thus, the mean exercise intensities were $56 \pm 5\%\ VO_{2\text{max}}$ during the initial EHT and $49 \pm 5\%\ VO_{2\text{max}}$ during the final EHT. These results from EHT (36.5 °C) are shown in Table 4.

The change of rectal temperature ($\Delta T_{re}$) and change of skin temperature ($\Delta T_{sk}$) during the EHT were altered by 7 – 8 wk of HAPT, but there were no differences between groups in the change of rectal or mean skin temperature. During the initial EHT, mean $\Delta T_{re}$ ($+1.2 \pm 0.4 \, ^\circ \text{C}$) and $\Delta T_{sk}$ ($+0.8 \pm 0.8 \, ^\circ \text{C}$) were significantly different ($P < 0.005$ and $P < 0.00001$, respectively; $n = 34$) from the $\Delta T_{re}$ ($+1.0 \pm 0.3 \, ^\circ \text{C}$) and $\Delta T_{sk}$ ($-0.3 \pm 0.8 \, ^\circ \text{C}$) measured during the second EHT.
Table 5 contains plasma measurements from EHT blood samples. The main effect (between-group) was not significant for any variable. However, as a result of HAPT, plasma lactate (at post-EHT) significantly decreased ($P < 0.05$).

Diastolic BP rose during the initial EHT ($P < 0.0005$) and during the final EHT ($P < 0.002$) in all groups. The grand means for diastolic values were: initial EHT (pre, 73 ± 10 mmHg; post, 63 ± 10 mmHg), second EHT (pre, 72 ± 8 mmHg; post, 65 ± 8 mmHg).

Systolic BP was similar during the initial EHT (baseline versus end of exercise, $P > 0.05$), but rose during the second EHT ($P < 0.0005$) in all groups. The grand means ± SD for systolic values were: initial EHT (pre, 103 ± 12 mmHg; post, 108 ± 17 mmHg), second EHT (pre, 101 ± 7 mmHg; post, 115 ± 10 mmHg). No differences due to treatment group or training were detected.

Sweat Sensitivity. Local sweat rate measurements (22.5°C environment) are illustrated in Figure 2. During HAPT, the sweat onset temperature decreased significantly in ORAL (before HAPT, 37.5 ± 0.2°C; after HAPT, 37.2 ± 0.4°C; $P < 0.05$), but not EU-OV (before HAPT, 37.5 ± 0.2°C; after HAPT, 37.4 ± 0.2°C) or DEPO (before HAPT, 37.7 ± 0.1°C; after HAPT, 37.7 ± 0.2°C) groups. This resulted in a statistically lower threshold of sweat onset temperature in the ORAL group compared to the DEPO group (post-HAPT; $P < 0.05$). Sweat sensitivity (i.e. the slope of the linear regression of rectal temperature plotted versus local sweat rate; linear portion of continuous measurements; Figure 2) was statistically similar between treatment groups, and was not altered by HAPT.

Discussion
This investigation was designed to evaluate the differential effects of oral and injectable contraceptive therapies on HAPT adaptations during 7 – 8 wk of supervised exercise sessions. Consistent with initial hypotheses, (a) the stress of HAPT did not disrupt the menstrual cycle length/phase characteristics, ovulation, or plasma hormone concentrations of EU-OV; and (b) no between-group differences (P>0.05) existed for rectal and skin temperatures, metabolic, cardiorespiratory (baseline, final, delta; during EHT), muscular endurance, or body composition variables. In addition, controlled measurements of fitness, body composition, VO₂max (Table 3), and exercise-heat tolerance (EHT; Figure 2, Tables 4 and 5) demonstrated that the subjects in all treatment groups exhibited physical fitness and heat acclimation adaptations.

The physiological adaptations to physical training in a cool environment are similar to those experienced in a hot environment, with a few minor exceptions (4, 16). The nature of HAPT (i.e., which included both physical training in a cool-cold outdoor environment and exercise-heat stress in an environmental chamber) made it difficult to determine which adaptations were stimulated by physical training and which by heat exposure exclusively, but some adaptations may be attributed. For example, specific physical training improved muscular endurance (i.e., situps, pushups), endurance running time, and VO₂max (Table 3). The low-intensity heat exposures of HAPT likely would not have affected these variables, but likely resulted in reduced submaximal VO₂ (i.e., specific to exercise-heat acclimation, not physical training) (3, 4). A future study, incorporating a longitudinal cross-over design (i.e., the most effective way to separate heat acclimation adaptations from those due solely to physical training [(4)])), would clarify this issue.

As the number of opportunities for women to participate in exercise and sport continues to increase, the number of women involved in exercise and sport programs continues to rise in the United States. As a result of this increased participation in sport by
women, the incidence of menstrual cycle disturbances also has increased (10, 18, 30). However, our observations of 36 healthy young women, who participated in 7 – 8 wk of HAPT, indicated that the menstrual status of the EU-OV group remained unchanged and consequently no abnormalities of reproductive hormone concentrations, and menstrual cycle phase characteristics, were observed.

**Blood Analyses.** Although plasma volume (PV) typically expands during the initial 5 - 10 days of heat acclimation (3), the dynamics of fluid shifts in women (Table 5) within the EHT test were not affected by HAPT; this supports previous observations of women, before and after exercise-heat acclimation (cycling exercise, 45°C environment) (20). Similar to PV, plasma glucose concentration was not affected by HAPT (Table 5). To our knowledge, no previous study has reported plasma glucose concentration adaptations in women during heat acclimation.

Regarding plasma lactate, a significant main effect (time, $P < 0.05$) was detected at the end of EHT. Thus, despite exercising in the heat 28.6 min longer during the second controlled test (before HAPT, 77.7 ± 9.2 min; after HAPT, 106.3 ± 41.7 min; all groups combined), at the same absolute treadmill speed, plasma lactate concentration decreased. This finding likely arose from either the lower relative exercise intensity (i.e., due to an increased VO$_{2\text{max}}$ and improved exercise efficiency subsequent to HAPT), or enhanced lactate clearance (not measured), during the final EHT (7).

**Thermal Responses.** The ORAL group experienced a decrease of the threshold rectal temperature for the onset of sweating (i.e., within group, NA versus ACCL; see * symbol in Fig. 3) as a result of HAPT. This adaptation also was observed in women who completed 60 days of physical training with no contraception therapy (2). However, similar oral contraceptive preparations (i.e., combined estrogen and progestin) have been shown to
increase the sweating threshold of young women, in a manner similar to the endogenous hormones of the luteal phase (15, 21). Thus, future studies are required to clarify why a significant mean difference of 0.6°C occurred between the threshold temperatures of ORAL and DEPO (Fig. 3, see ** symbol). Such studies should include women who undertake physical training or heat acclimation programs.

Sweat sensitivity (i.e., the slope of each regression line in Fig. 3; the ratio of Δsweat rate / ΔT_{re}), measured locally over the scapula, was not different between treatment groups, before or after HAPT. This finding is similar to observations published by Charkoudian and Johnson (15) and Grucza et al. (21), which involved the chronic use of female reproductive hormones and changes in the threshold temperatures for effector responses with no change in sweat sensitivity. Further, when estrogen supplementation was administered for only three days, Chang et al. (14) observed no adaptation of either the threshold temperature or the sweat sensitivity. At present, the evidence suggests that sweat sensitivity is not influenced by female reproductive hormones.

Sweat production is controlled by the anterior hypothalamus, which integrates afferent T_{re} and T_{sk} impulses and regulates effector responses, including eccrine sweat gland secretion and skin blood flow (27). Final SR_{wb} did not change as a result of either HAPT or contraceptive type (i.e., both main effects P > 0.05, Table 4). This phenomenon was recognized by Henane (22) as one result of the reduced body temperature that occurs with heat acclimation. Because final T_{re}, ΔT_{re}, and ΔT_{sk} decreased during HAPT, the neural drive for sweat production decreased.

Summary. Numerous long-term adaptations occurred during HAPT (i.e., significant main effect of time), in response to HAPT sessions performed by 36 young women. However, the differences of adaptations in ORAL, EU-OV and DEPO were few,
small in magnitude, and did not impart superior physical fitness or heat acclimation to any treatment group. Perhaps the most interesting between-group differences involved the threshold temperatures for local sweating. This threshold decreased in ORAL as a result of HAPT ($P < 0.05$), suggesting that an EE-progestin formulation may lower the hypothalamic set-point; also, the use of depot MPA did not induce a significant change of this threshold during HAPT. Although some pharmacologic agents may alter sweat rate or reduce cardiac contractility during exercise in a hot environment (25), the use of contraceptives in the present investigation (ORAL and DEPO versus EU-OV) did not impair or improve the ability of women to complete 7 – 8 wk of strenuous HAPT.
Acknowledgements

The authors gladly acknowledge the technical contributions of the following individuals: Dean Aresco, Lynn Bairos, Timothy Bilodeau, David Blair, Douglas R. Bolster, Valerie A. Collins, Stephen L. Gaffin, Jorge Herrera, Jennifer Holub, Gregory Kane, Brian E. Miller, Jennifer Ormerod, and Melissa Roti. Physician Jeffrey Anderson served as the medical monitor for this investigation.

Grants

This research was funded by a grant from the U.S. Army Medical Research and Materiel Command, Fort Detrick, MD, under the auspices of the Defense Women's Health Research Program.
References


Figure Legends

Figure 1. Experimental measurements taken during all exercise-heat tolerance tests (EHT), before and after the 7 – 8 wk heat acclimation-physical training (HAPT) program. If the experiment was halted prior to 90 min, measurements A, B, D, E and F were taken just prior to the end of exercise. Abbreviations: EHT, exercise-heat tolerance test, conducted before and after HAPT; RH, relative humidity; $T_{sk}$, 3-site mean weighted skin temperature; HR, heart rate; $T_{re}$, rectal temperature; RPE, rating of perceived exertion; VO$_2$, oxygen consumption.

Figure 2. Relationships between local sweat rate and rectal temperature ($T_{re}$), before and after heat acclimation-physical training (HAPT), in ORAL, EU-OV and DEPO groups. Slopes (i.e., statistical regressions of the linear segment of continuous local sweat rate measurements) represent the mean of all subjects and were statistically similar (P>.05). Symbols: *, significant difference (across time, P < 0.05) of sweat onset temperature (ORAL only) due to HAPT; **, significant between-group difference of sweat onset temperature (ORAL < DEPO, P < 0.05) at the post-HAPT time point only.
Table 1. Synthetic steroid content of the oral contraceptives that were voluntarily taken by women in the ORAL group (n = 15).

<table>
<thead>
<tr>
<th>Estrogen (mg)</th>
<th>Progestin (mg)</th>
<th>Oral Contraceptive Trade Name</th>
<th>Number of Subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>EE, 0.035 mg</td>
<td>Norethindrone, 1.00 mg</td>
<td>Ortho-Novum 10/11™</td>
<td>3</td>
</tr>
<tr>
<td>EE, 0.035 mg</td>
<td>Norethindrone, 0.50, 0.75, 1.00 mg</td>
<td>Ortho-Novum 7/7/7™</td>
<td>3</td>
</tr>
<tr>
<td>EE, 0.035 mg</td>
<td>Norgestimate, 0.25 mg</td>
<td>Ortho-Cyclen™</td>
<td>2</td>
</tr>
<tr>
<td>EE, 0.035 mg</td>
<td>Norgestimate, 0.18, 0.22, 0.25 mg</td>
<td>Ortho-TriCyclen™</td>
<td>3</td>
</tr>
<tr>
<td>EE, 0.030 mg</td>
<td>Desogestrel, 0.02 mg</td>
<td>Marvelon 28™</td>
<td>2</td>
</tr>
<tr>
<td>EE, 0.030 mg</td>
<td>Gestodene, 0.75 mg</td>
<td>Femodene™</td>
<td>2</td>
</tr>
</tbody>
</table>

Note: the values in columns 1 and 2 indicate the amount of ethinyl estradiol (EE) or progestin consumed for 21 days, followed by 7 days of placebo pills or no pills.
Table 2. Endogenous blood variables (mean ± SD) related to reproductive function, measured at rest, before (NA) and after (ACCL) the HAPT program.

<table>
<thead>
<tr>
<th>Treatment Group (n)</th>
<th>E$_2$ (pmol·L$^{-1}$)</th>
<th>P$_4$ (ng·ml$^{-1}$)</th>
<th>PRL ($\mu$g·L$^{-1}$)</th>
<th>SHBG (nmol·L$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ORAL (15)</td>
<td>120.8 ± 18.0</td>
<td>0.9 ± 0.2</td>
<td>14.7 ± 2.1</td>
<td>157.1 ± 14.5 *</td>
</tr>
<tr>
<td></td>
<td>106.8 ± 11.7</td>
<td>0.9 ± 0.1</td>
<td>15.2 ± 2.1</td>
<td>180.0 ± 20.6 *</td>
</tr>
<tr>
<td>EU-OV (14)</td>
<td>112.7 ± 11.4</td>
<td>0.8 ± 0.1</td>
<td>13.8 ± 2.0 †</td>
<td>44.3 ± 3.0</td>
</tr>
<tr>
<td></td>
<td>96.9 ± 9.5</td>
<td>0.9 ± 0.1</td>
<td>18.4 ± 2.2 †</td>
<td>45.4 ± 4.0</td>
</tr>
<tr>
<td>DEPO (7)</td>
<td>87.7 ± 11.4</td>
<td>0.8 ± 0.2</td>
<td>18.3 ± 5.2</td>
<td>39.9 ± 7.7</td>
</tr>
<tr>
<td></td>
<td>82.2 ± 5.9</td>
<td>0.8 ± 0.1</td>
<td>22.8 ± 8.8</td>
<td>39.8 ± 7.0</td>
</tr>
</tbody>
</table>

Blood samples were drawn as follows: EU-OV, during the early follicular phase (days 2 – 5); ORAL, during days 2 – 5 of the placebo phase of the pill pack; DEPO, during days 2 – 5 of an arbitrary 28-day period, as assigned by investigators.

Symbols: *, significant (P < 0.05) between-group difference (ORAL versus EU-OV, ORAL versus DEPO); † - significant (P < 0.05) difference across time (NA versus ACCL).

Abbreviations: HAPT, heat acclimation-physical training program; NA, not heat acclimated, before HAPT; ACCL, heat acclimated, after HAPT; ORAL, oral contraceptive group; EU-OV, eumenorrheic-ovulatory group; DEPO, Depo Provera™ group; E$_2$, estradiol; P$_4$, progesterone; PRL, prolactin; SHBG, sex hormone binding globulin.
Table 3. Physical training and body composition variables (mean ± SD), measured before (NA) and after (ACCL) HAPT.

<table>
<thead>
<tr>
<th>Group</th>
<th>Pushups (per 60 s)</th>
<th>Situps (per 60 s)</th>
<th>4.6-km Run Time (min)</th>
<th>Body Mass (kg)</th>
<th>Body Fat (%)</th>
<th>Fat-Free Mass (kg)</th>
<th>VO$_{2\text{max}}$ (L·min$^{-1}$)</th>
<th>VO$_{2\text{max}}$ (ml·kg$^{-1}$·min$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NA</td>
<td>19 ± 6</td>
<td>45 ± 9</td>
<td>53 ± 10</td>
<td>40.1 ± 12.4</td>
<td>± 5.8</td>
<td>± 12.4 ± 6.8</td>
<td>65.8 ± 26.0</td>
<td>26.0 ± 5.7</td>
</tr>
<tr>
<td>ACCL</td>
<td>76 ± 20</td>
<td>76 ± 21</td>
<td>71 ± 12</td>
<td>62.8 ± 5.9</td>
<td>± 8.8</td>
<td>± 5.9 ± 5.9</td>
<td>63.2 ± 26.5</td>
<td>24.6 ± 4.7</td>
</tr>
<tr>
<td></td>
<td>± 19 ± 10</td>
<td>± 10 ± 12</td>
<td>± 9.1 ± 5.4</td>
<td>± 10.5 ± 5.6</td>
<td>± 7.6 ± 3.1</td>
<td>± 11.1 ± 5.6</td>
<td>71.6 ± 31.8</td>
<td>26.3 ± 4.7</td>
</tr>
<tr>
<td>ORAL</td>
<td>± 19 ± 17</td>
<td>± 10 ± 13</td>
<td>± 9.1 ± 5.4</td>
<td>± 10.5 ± 5.6</td>
<td>± 7.6 ± 3.1</td>
<td>± 11.1 ± 5.6</td>
<td>71.6 ± 31.8</td>
<td>26.3 ± 4.7</td>
</tr>
<tr>
<td>EU-OV</td>
<td>± 26 ± 10</td>
<td>± 12 ± 11</td>
<td>± 9.1 ± 5.4</td>
<td>± 10.5 ± 5.6</td>
<td>± 7.6 ± 3.1</td>
<td>± 11.1 ± 5.6</td>
<td>71.6 ± 31.8</td>
<td>26.3 ± 4.7</td>
</tr>
<tr>
<td>DEPO</td>
<td>± 17 ± 13</td>
<td>± 10 ± 12</td>
<td>± 9.1 ± 5.4</td>
<td>± 10.5 ± 5.6</td>
<td>± 7.6 ± 3.1</td>
<td>± 11.1 ± 5.6</td>
<td>71.6 ± 31.8</td>
<td>26.3 ± 4.7</td>
</tr>
</tbody>
</table>

All variables, except the 4.6 km outdoor run, were measured in a 22.5°C environment. Symbols: * - significant main effect (across time, P < 0.05) during HAPT. No between-group differences existed for any variable. Abbreviations: NA, not heat acclimated, before HAPT; ACCL, heat acclimated, after HAPT; ORAL, oral contraceptive group; EU-OV, eumenorrheic-ovulatory group; DEPO, Depo Provera™ group; VO$_{2\text{max}}$, maximal aerobic power.
Table 4. Physiologic variables (mean ± SD), measured during EHT (36.5 °C), before and after HAPT.

<table>
<thead>
<tr>
<th>Group</th>
<th>Exercise Time (min)</th>
<th>SR\textsubscript{wb} (ml·kg\textsuperscript{-1}·h\textsuperscript{-1})</th>
<th>HR (beats·min\textsuperscript{-1})</th>
<th>T\textsubscript{re} (°C)</th>
<th>T\textsubscript{sk} (°C)</th>
<th>RPE</th>
<th>VO\textsubscript{2} (ml·kg\textsuperscript{-1}·min\textsuperscript{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>NA</td>
<td>ACCL</td>
<td>NA</td>
<td>ACCL</td>
<td>NA</td>
<td>ACCL</td>
<td>NA</td>
<td>ACCL</td>
</tr>
<tr>
<td>ORAL</td>
<td>45.6 ± 16.1</td>
<td>76.0 ± 19.2</td>
<td>11.4 ± 3.9</td>
<td>11.9 ± 1.5</td>
<td>178 ± 11</td>
<td>155 ± 14</td>
<td>38.4 ± 0.3</td>
</tr>
<tr>
<td>EU-OV</td>
<td>45.6 ± 19.3</td>
<td>75.3 ± 12.8</td>
<td>14.3 ± 5.1</td>
<td>10.5 ± 3.1</td>
<td>179 ± 10</td>
<td>155 ± 13</td>
<td>38.6 ± 0.4</td>
</tr>
<tr>
<td>DEPO</td>
<td>34.0 ± 13.0</td>
<td>67.1 ± 15.2</td>
<td>14.5 ± 4.1</td>
<td>13.9 ± 2.9</td>
<td>186 ± 12</td>
<td>156 ± 8</td>
<td>38.4 ± 0.5</td>
</tr>
</tbody>
</table>

Note: to provide a controlled comparison, values for HR, T\textsubscript{re}, T\textsubscript{sk}, RPE and VO\textsubscript{2} during the final EHT (ACCL, after HAPT) were recorded at the time that the initial EHT test (NA, before HAPT) ended. Symbols: * - significant (P < 0.05) difference for main effect across time; no between-group differences existed. Abbreviations: EHT, exercise-heat tolerance test; HAPT, 7 – 8 wk heat acclimation-physical training program; ORAL, oral contraceptive; EU-OV, eumenorrheic-ovulatory; DEPO, Depo Provera\textsuperscript{TM}; SR\textsubscript{wb}, whole body sweat rate; HR, heart rate; T\textsubscript{re}, rectal temperature; T\textsubscript{sk}, mean weighted skin temperature (3 sites); RPE, rating of perceived exertion; VO\textsubscript{2}, submaximal oxygen consumption; NA, not acclimated, before HAPT; ACCL, acclimated, after HAPT.
Table 5. Plasma variables (mean ± SD) measured during EHT, before and after HAPT.

<table>
<thead>
<tr>
<th>Group</th>
<th>Plasma Glucose (mmol·L⁻¹)</th>
<th>Plasma Lactate (mmol·L⁻¹)</th>
<th>Change in PV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre Post Pre Post</td>
<td>Pre Post Pre Post</td>
<td>NA ACCL NA ACCL</td>
</tr>
<tr>
<td>ORAL</td>
<td>5.4 ± 0.7 6.0 ± 0.9</td>
<td>5.4 ± 0.7 6.0 ± 1.2</td>
<td>-2.4 ± 3.5</td>
</tr>
<tr>
<td></td>
<td>1.3 ± 0.4 1.8 ± 0.4</td>
<td>1.3 ± 0.4 1.5 ± 0.4</td>
<td>± 3.5 ± 4.4</td>
</tr>
<tr>
<td>EU-OV</td>
<td>5.6 ± 0.9 5.6 ± 0.5</td>
<td>5.4 ± 0.9 6.2 ± 0.9</td>
<td>-3.9 ± 4.7</td>
</tr>
<tr>
<td></td>
<td>1.3 ± 0.5 2.0 ± 0.5</td>
<td>1.3 ± 0.5 1.8 ± 0.7</td>
<td>± 4.9 ± 7.0</td>
</tr>
<tr>
<td>DEPO</td>
<td>5.5 ± 0.7 6.4 ± 0.6</td>
<td>5.4 ± 0.6 6.4 ± 1.5</td>
<td>-1.2 ± 3.3</td>
</tr>
<tr>
<td></td>
<td>1.1 ± 0.2 2.1 ± 0.3</td>
<td>1.1 ± 0.2 1.0 ± 0.7</td>
<td>± 4.2 ± 3.9</td>
</tr>
</tbody>
</table>

Symbols: * - significant main effect (across time, P < 0.05), NA-post versus ACCL-post only. Abbreviations: EHT, standardized exercise-heat tolerance test; HAPT, 7 – 8 wk heat acclimation-physical training program; PV, plasma volume; NA, not heat acclimated, before HAPT; ACCL, heat acclimated, after HAPT; Pre, before EHT exercise began; Post, immediately after EHT exercise ended; ORAL, oral contraceptive group; EU-OV, eumenorrheic-ovulatory group; DEPO, Depo Provera™ group.
Figure 1

24-h water restriction
Standing equilibration
Controlled EHT: walking 93.6 m·min⁻¹ at 5% grade in 36.5°C and 37% RH

Time (min)

K E Y:
A, body mass
B, blood pressure
C, CVC
D, Tsk
E, HR, Tres, RPE
F, blood sampling
G, VO₂
Figure 2

Before HAPT

After HAPT

Local Sweat Rate (mg·min⁻¹·cm⁻²)

EU-OV

**

*

ORAL

Rectal Temperature (°C)

DEPO