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ARTICLE

Effects of Zumba® and different walking workouts on female cortisol and DHEA-S production

Andrea Di Blasio[1], Serena Di Santo[1], Nunzia Lomonaco[1], Cesidio Giuliani[1], Ines Bucci[1], and Giorgio Napolitano[1]

Abstract

Purpose: Aims of our study were to characterize both heart rate and hormonal responses elicited by three different fitness disciplines: outdoor walking training, Zumba®, and walking training on magnetic treadmill. *Methods:* Fifteen healthy trained women (42 ± 7 yr) performed three different workouts conducted by the same instructor. Salivary samples were collected before and after each workout, at 11 p.m. and at 7 a.m. of the following day, while a beat-to-beat heart rate recording was executed during each workout. Saliva was also collected during a non-training day. Cortisol and dehydroepiandrosterone sulfate (DHEA-S) were measured in salivary samples. *Results:* Workouts have been shown able to affect physiological trend of LogCortisol according to intensity: as highest was workout intensity as highest was LogCortisol at 11 p.m. The same has been shown for LogDHEA-S except for outdoor walking training that has not been able to affect salivary LogDHEA-S. Time elapsed at both 60-69% maximal heart rate (HRmax; r=0.301, p=0.05) and 80-89% HRmax (r=-0.328, p=0.003) have been shown correlated with LogCortisol to LogDHEA-S ratio. *Conclusions:* The knowledge of acute and delayed hormonal effects of fitness disciplines is very important to optimize health promotion and maintenance of midlife trained women, near to menopausal transition. **Health & Fitness Journal of Canada 2015;8(4):14-24.**

Keywords: steroid hormones, cortisol to DHEA-S ratio, exercise intensity, heart rate, salivary sampling, women

From Endocrine Section, Department of Medicine and Aging Sciences, "G. d'Annunzio", University of Chieti-Pescara, Chieti, Italy. Email: andiblasio@gmail.com

Introduction

The importance of physical exercise practice for health, at any age, in both sexes and in several pathological conditions, is so widely accepted that physical exercise prescription are increasing at all ages (Garcia, 2014). Among adults, two main different scenarios are possible: participation to fitness clubs activities or participation to outdoor activities. In both cases, participants chose between group or individual activities, that could be supervised or not supervised. Even if the choice of each participant is driven by different causes (Biddle and Mutrie, 2008), whose knowledge is important to secure adherence to physical exercise practice but also to correctly plan physical exercise offer (Thompson, 2014), the most important thing is the choice of the proper activity according to own physio-pathological and psychological characteristics. As female physiology has an intrinsic risk factor for health, named menopause, it is important to focus our attention to midlife women in order to apply an effective primary prevention of chronic diseases because physiological menopause is not a sudden event but it is preceded by a preparatory phase. This phase is named pre-menopause and lasts an average 5 yr during which there is a progressive decline in ovary function and a progressive onset of its consequences, such as a progressive increase of waist circumference, body fat, total and low density cholesterol, together with plasma triglycerides, citing the most common and simply measurable sequelae (Sternfeld and Dugan, 2011). A natural way to reduce the entity and counteract the major part negative sequelae of menopause is physical exercise that powerful is greater as soon it is started (Sternfeld and Dugan, 2011). Due to the hormonal trend during both pre-menopause and menopause, characterized by a cortisol increase and anabolic hormones decrease, determining a chronic catabolic state, physical exercise should be chosen, programmed and practiced in order to counteract this situation. Growing literature is coming about the important role of cortisol to dehydroepiandrosterone sulfate (DHEA-S) ratio both in female and male health (Phillips et al, 2010): as lower is the ratio as higher is health because a lower ratio reflects, for example, a low catabolic and pro-inflammatory state, due to higher plasma cortisol and lower DHEA-S, while a higher ratio reflects an anabolic and immunostimolant state, due to lower cortisol and higher DHEA-S. Even if physical exercise has been shown able to regulate both cortisol and DHEA-S production, literature underlines that physical exercise characteristics (i.e. type, duration and intensity) can differently stimulate hormonal production, according to their organization (Kraemer and Ratamess, 2005; Constantini and Hackey, 2013). Therefore, starting from the study of Daley and colleagues (2011), investigating exercise preferences of menopausal women, and from the studies of Thompson and colleagues (2014), investigating fitness trends and fads, aim of our study was to verify whether three of the most practiced disciplines among premenopausal and menopausal women (i.e., Zumba®) walking workout on magnetic treadmill (WWMT), and outdoor walking workout (OuWW) satisfy the hormonal necessities of women approaching menopause.

Materials and methods

Participants

Fifteen healthy non-smokers trained eumenorroic women (42 ± 7 yr) were recruited and participated to the study completing it. Participants were recruited at a Fitness Club in Chieti, Italy among the costumers who were free from any diseases and were accustomed with the experimented workouts and functional movements. All of the participants provided written informed consent. The research was conducted respecting the ethical principles of the Declaration of Helsinki.

Study Design

All participants underwent a careful clinical examination, anthropometry, body composition assessment, and preliminary endurance testing before the execution of the experimental workouts. Then, each workout was tested in a different week, while two resting days anticipated and followed the testing days. At each workout, a battery of measurements and withdrawals was applied to detect endocrine and cardiac responses elicited by the workout. Two exercise specialists supervised each workout that was conducted by the same instructor. During all the phases of study, the laboratory and gym conditions were controlled for temperature (21–23°C) and humidity (50%) (ACSM, 2008).

Clinical examination, anthropometry and body composition

Clinical examination was executed by a medical doctor specialized in sports medicine to exclude any diseases. A first level anthropometrist of the International Society for the Advancement of Kinanthropometry measured body weight and stretched stature and performed electrical bioimpedance analysis according to international guidelines (ACSM, 2008; Marfell-Jones et al., 2006). A stadiometer with a balance-beam scale (Seca 220, Seca, Hamburg, Germany) and a foot-to-foot 50 kHz frequency bioelectrical impedance scale (BC-420MA, Tanita, Tokyo, Japan) were used.

Preliminary testing

Preliminary testing for endurance exercises included the assessment of subjects' maximal heart rate through a graded stress test (i.e. Bruce protocol) on a treadmill (770 CE, RAM, Padua, Italy), under continuous electrocardiogram monitoring and step-by-step blood pressure measurements [ACSM]. The graded stress test was also used to confirm the cardiovascular eligibility of each participant and to estimate his/her maximal aerobic power using the following formula (ACSM, 2008):

$VO_2max\ (mL \cdot kg^{-1} \cdot min^{-1}) = 2.94 \times T + 3.74$

Measurements and withdrawals

Participants, that always trained at the same hour, presented in the gym at 6:30 p.m. and started the training, 2.5 hr after a standardized meal, at 7:00 p.m., without having performed maximal muscle exertion, sexual intercourses, and abstaining from stimulants and alcohol from2 days before to the experimental days and until 9:00 a.m. of the following day. The meal was lower to 400 kcal (Compher et al., 2006) and consisted of 33 cl of water, 35 cl of orange juice and one 30 g energy bar (Power Sport Double Use, Enervit, Milan, Italy). Before to start each workout, in a standardized room (ACSM, 2008), each subject wore a Polar Loop (Polar, Kempele, Finland), settled to record heart rate at each second of the training. After that, a salivary sample was collected using Salimetrics Oral Swab (Salimetrics Europe, Suffolk, UK). Before to start the workout, each participant did a standardized warm-up of 10 minutes under the supervision of the exercise specialists. Salivary sample was also collected at the end of the workout, at 11:00 p.m. of the same day and at 7:00 a.m. of the day following the workout. Participant avoided stimulants and alcohol intake during the experimental days and avoided food intake during the 2 hr before each collection. Each participant recorded the dinner of the first workout they experimented in order to have the same dinner at each experimental day. We did not furnish indication about the quantity that was *ad libitum*.

In order to better determine the delayed workout-elicited endocrine responses, salivary samples were also collected during a non-training (CONTROL) day, respecting the same standardization used for training days collections. CONTROL day salivary collections were executed at 7:00 p.m., 8:00 p.m., 11:00 p.m. and at 7:00 a.m. of the following day.

Analytical methods

Salivary samples were collected using Salimetrics Oral Swab (Salimetrics Europe, Suffolk, UK), i.e. a small pad, absorbent and non-toxic, passed within the oral cavity for 2-3 minutes before being placed inside a tube labeled. The samples were refrigerated within 30 minutes and frozen at -20° C within 2 hr of collection. The day of the assay, samples were thawed and centrifuged for

15 minutes at 3,000 rpm to extract saliva and remove the mucin and the swab was then discarded. The assays were performed using the following salivary assay kits: Salivary DHEA-SEIA kit and High Sensitivity Salivary Cortisol EIA Kit (Salimetrics Europe, Suffolk, UK). The intra-assay coefficient of variation of cortisol was 4.6% while that of DHEA-S was 7.25%.

Experimental workouts: characteristics

Each workout had the same duration and organizational characteristics: 5-10 minutes of warm-up, 40-50 minutes of central part and 5-10 minutes of cool-down. Zumba® workout was conducted according to principles of Zumba® certification scheme, while WWMT was conducted according to principles of Walking Program® certification scheme. The Walking Program® workout was conducted using magnetic treadmills WALKER Plus (Fitness Project srl, Padova, Italy) characterized by a fixed slope of 15%. Outdoor walking workout was conducted on urban route characterized by the presence of slopes not exceeding 3%.

Statistical analysis

The STATA 10 software (StataCorp LP, College Station, USA) was used for statistical analysis. The data were tested for normality and cortisol and DHEA-S were logarithmically transformed because found non-normally distributed. Descriptive statistic was used to characterize study participants. The Kruskal-Wallis test was used to compare workouts intensity distribution and post-hoc analysis, i.e. the Mann-Whitney test, were performed to better know differences of each discipline with others. In order to know whether the experimented workouts differently influenced the physiological trend of cortisol and DHEA-S production, and of their ratio, analysis of variance, with repeated measures for the factor 'time' (RM-ANOVA), was used to investigate the presence of*time,* or *disciplines* × *time* effect on hormones production. RM-ANOVA was performed comparing the values recorded during training days + following morning with those recorded during CONTROL day + following morning. When a *disciplines* × *time* effect was found, new RM-ANOVA, including only two sampling periods each time, were performed, to better know hormonal effects of different workouts. Partial correlations were performed to establish if heart rate variables were correlated with salivary hormones collected at 11:00 p.m. and 7:00 a.m., independently from their level recorded, in the same hour, during CONTROL day. Statistical significance was set at $p \leq 0.05$ and data were presented as means ±standard deviations also when non-parametric test was performed to give the possibility to the readers to know the values of each variable.

Results

One participant dropped out for personal motivations. Table 1 shows basal characteristics of participants that completed the study: they were meanly normal-weight, indeed majority of our subjects was normal-weight while some participants were overweight. Mean estimated aerobic fitness of participants was "above the average" of general population, stratified for gender and age, while the distribution ranged from "average" and "good categories" (Shvartz and Reibold, 1990).

Table 1: Basal characteristics of participants.

	Mean ± SD n = 14
Age (yr)	43 ± 7
Body Mass Index (kg·m^{-2})	23.6 ± 3.6
Fat mass (%)	26.7 ± 7.9
HR_{max} (bpm)	178.0 ± 4.0
VO_2max (mL·kg^{-1}·min^{-1})	37.9 ± 6.3

Note: HRmax = maximal heart rate; VO_2max = maximal aerobic power

Table 2 shows cardiac engagement of the experimented disciplines. According to Kruskall-Wallis test results, they differed for time elapsed at different intensities. Post-hoc analysis confirmed that OuWW elicited the lowest cardiac engagement respect to others workouts, indeed, the major part of the workout was elapsed at intensity comprised between 60 to 69 % of HR_{max}, while WWMT elicited the highest cardiac engagement with more than half session elapsed at intensities major than 80% of HR_{max}.

A RM-ANOVA confirmed that the experimented disciplines significantly affected the physiological trend of LogCortisol, LogDHEA-S and LogCortisol to LogDHEA-S ratio showing both *time* (LogCortisol: F=43.365, p<0.001; LogDHEA-S: F=20.610, p<0.001; LogCortisol to LogDHEA-S ratio: F=5.284, p=0.003), and *disciplines* × *time* effect.

Observing LogCortisol results, post-hoc analysis, performed to deep the knowledge of the *disciplines* × *time* effect (F=7.904, p<0.001), underlined the significant different trend of salivary LogCortisol during workouts and following days, respect to non-training days: the increase at 11:00 p.m. and decrease from 11:00 p.m. to 7:00 a.m. characterize workouts and following days while opposite trend was recorded during non-training days (Table 3 and Figure 1).

Observing LogDHEA-S results, post-hoc analysis, performed to deep the knowledge of the *disciplines* × *time* effect (F=4.548, p<0.001), underlined the significant different trend of salivary LogDHEA-S during workouts and following days, respect to non-training days: as highest was workout intensity as highest was salivary LogDHEA-S at 11:00 p.m. and lowest at 7:00 a.m. while the lowest value of salivary LogDHEA-S at 11:00 p.m. and its highest value were recorded during non-training days. Walking training did not elicit a significant different trend of salivary LogDHEA-S respect to non-training days

Table 2: Cardiac engagement of the experimented disciplines.

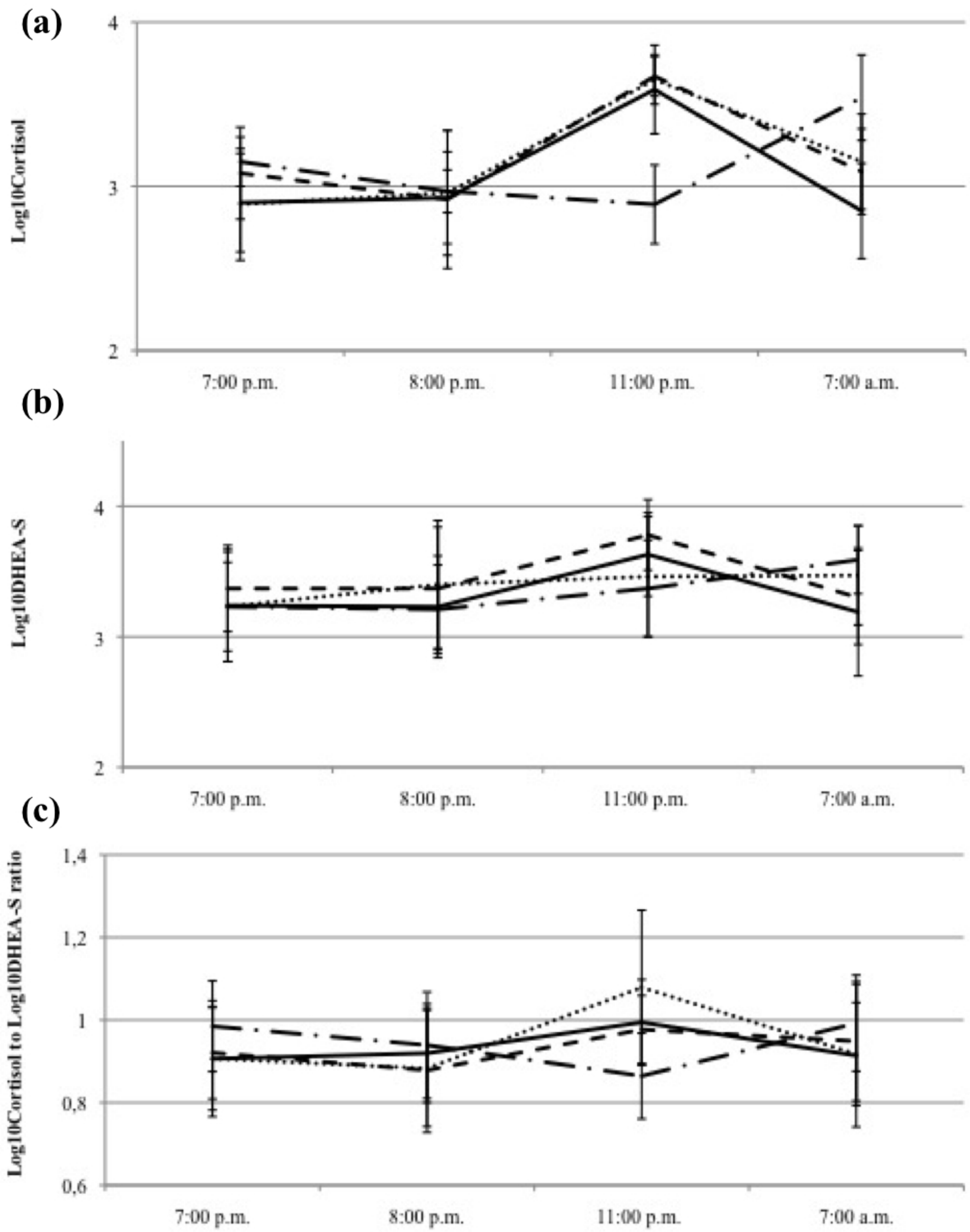
	Zumba®	WWMT	OuWW	*p*
R-HR_{max}(bpm)	150.14±11.33	160.71±13.34**	141.38±27.70°§	0.003
R-HR_{min} (bpm)	68.64±8.93	75.85±15.88	78.84±3.95°°	0.02
Time 60-69% HR_{max} (%)	17.00±0.01	19.59±8.17	61.63±26.82°°§§	<0.001
Time 70-79% HR_{max} (%)	28.05±3.96	18.51±3.34**	25.48±11.36°§§	<0.001
Time 80-89% HR_{max} (%)	50.21±16.10	50.39±9.39**	9.30±15.81°°§§	<0.001
Time ≥90% HR_{max} (%)	4.53±12.79	15.09±18.59**	3.60±6.87§§	<0.001

Note: WWMT, walking workout on magnetic treadmill; OuWW, outdoor walking workout; R-HR, recorded heart rate during workout; Time xx-xx% HR_{max}, time of the workout elapsed at intensity comprised between xx and xx of the HR_{max} expressed as percentage of the duration of the workout; **, post-hoc analysis statistical significance (i.e. p≤0.01) regarding differences between Zumba® and WWMT; °°, post-hoc analysis statistical significance (i.e. p≤0.01) regarding differences between Zumba® and OuWW; °, post-hoc analysis statistical significance (i.e. p≤0.05) regarding differences between Zumba® and OuWW; §§, post-hoc analysis statistical significance (i.e. p≤0.01) regarding differences between WWMT and OuWW; §, post-hoc analysis statistical significance (i.e. p≤0.05) regarding differences between WWMT and OuWW.

while Zumba® and WWMT did. On the contrary, Zumba® and WWMT did not elicited different trends of salivary LogDHEA-S (Table 3 and Fig. 1).

Observing LogCortisol to LogDHEA-S ratio results, post-hoc analysis, performed to deep the knowledge of the *disciplines* × *time* effect (F=2.976, p=0.003),

Figure 1: Hormonal trends.



Legend: continue line, Zumba® workout; dashed line, WWMT, walking workout on magnetic treadmill; dotted line, OuWW, outdoor walking workout; alternated dashed-dotted line, CONTROL day.

underlined that experimented workouts inverted the physiological trend the considered ratio both at 11:00 p.m. and at 7:00 a.m. of the day following the training (Table 3 and Fig. 1).

Partial correlation analysis showed that LogCortisol to LogDHEA-S ratio recorded at 11:00 p.m. of the workouts days was positively correlated with time engaged at 60-69% of HR_{max} (r=0.301, p=0.05) and negatively correlated with time engaged at 80-89% of HR_{max} (r=-0.328, p=0.003).

The most important result of our research is that in physical exercise prescription/choice, for women approaching the premenopause, it is very important to consider not only discipline but also its intensity in order to offer to practisers a physical exercise contrasting the natural decrease of anabolic hormones and increase of cortisol.

As showed for high-intensity interval resistance workouts in men (Di Blasio et al., 2016) also OuWW, Zumba® and WWMT are able to affect physiological hormonal production after workout conclusion in midlife women. Specifically, both cortisol and DHEA-S salivary trends have been shown affected by workouts, even if differences according to workout intensity are present. Indeed, as highest was workout intensity as highest was salivary LogCortisol response after exercise session: all workouts significantly inverted the physiological trend of salivary LogCortisol at 11 p.m., determining its increase instead of its decrease, respect to 7 p.m. sampling. To be more analytic about workouts differences, while Zumba® and WWMT did not elicited significant different responses, they elicited significant highest responses respect to OuWW, even if differences between WWMT and OuWW trends were near to statistical significance (Table 3 and Fig. 1). According to the literature, exercise intensity is the key of the explanation of our results, indeed, workouts that are high in volume and moderate to high in intensity elicit greatest lactate and cortisol production, with cortisol response more prominent as intensity increases (Hill et al., 2008). Even if lactate production was not investigated, to couple the study of estimated aerobic fitness of participants, allowing estimating also their anaerobic threshold (Sharkey and Gaskill, 2007), with the analysis of heart rate distribution of each workout, allowed detecting discipline with the greatest anaerobic component. Among the considered disciplines, OuWW elicited the lowest time elapsed at intensities major than 69% of HR_{max}, while WWMT elicited the highest time elapsed at intensities major than 79% of HR_{max}. Heart rate distribution analysis confirmed the moderate-intensity characteristic of OuWW, executed without slopes major than 3%, and high-intensity characteristic of both Zumba® and WWMT, conferring to WWMT the characteristic of highest intense workout, among those investigated, even if, in our case, there is only a little difference between Zumba® and WWMT (Table 2). Even if our study did not add important data about heart rate characterization of walking training, that is a submaximal workout, it adds important data about Zumba® and WWMT characterization: high-intensity disciplines alternating high-intensity periods to lower intensity periods.

The same trend, showed for LogCortisol, has been shown for salivary LogDHEA-S, presenting an interesting variation respect to salivary LogCortisol results: indeed, on the contrary of that observed for LogCortisol, OuWT has not been shown able to elicit a different trend of salivary LogDHEA-S respect to resting days. This result is in line with findings of both Giannopoulou and colleagues (2003) and Copeland and colleagues (2002) respectively finding 30 and 40 minutes of submaximal exercise not enough to elicit post-exercise DHEA-S increase. Our results suggest that exercise intensity have a key role also for salivary LogDHEA-S level and that it is necessary to reach a minimum intensity for a minimum time to allow its increase. As OuWW has been shown the discipline with the lowest anaerobic component it is allowed to hypothesize that it is necessary to sufficiently exceed anaerobic threshold to adequately stimulate DHEA-S production. Even if when we analyzed the LogCortisol to LogDHEA-S ratio trends and we did not observe significant differences among workouts, results of partial correlation gave important data about the relationship between exercise intensity and LogCortisol to LogDHEA-S ratio after the training: as highest/lowest was exercise intensity as lowest/highest was salivary LogCortisol to LogDHEA-S ratio 4 hr after the training.

Taken together hormonal results, our study gives important information for exercise choice and planning in women, especially in women approaching menopause, or being in menopause, in order to counteract the unfavourable physiological hormonal trend. Indeed, knowing the emerging important role of a low LogCortisol to LogDHEA-S ratio for female health (Phillips et al., 2010), it is important to know that walking training, without slopes higher than 3%, it is not able to stimulate DHEA-S production allowing an high LogCortisol to LogDHEA-S ratio state persisting till 4 hr after the training. Therefore, in order to elicit healthy hormonal pattern, on the contrary of that normally happens, women should not practice only an aerobic training similar to OuWW but they should couple it with resistance exercises (Copeland et al., 2002; Phillips et al., 2010; Copeland et al., 2004) or change the typology of aerobic training choosing one discipline more able to increase DHEA-S levels, if participant does not particularly like resistance training.

The small sample size, together with estimation of aerobic fitness and anaerobic threshold of participants, is the main limitation of the study, therefore, the observed results must be considered not as a statement but as a starting point for further studies including higher sample size. However, the fact that the study was conducted on a group of expert participants and that each participant executed all of the experimented workouts is strength and can partially compensate the small sample size. Another limitation of the study is the absence of a completely controlled diet, even if, maintaining the ad libitum principle, standardizing pre-workout meal and demanding to participants to eat the same dinner, at each experimental day, could be sufficient to completely exclude the presence of dietary habits influences on our results. Also the absence of a randomization of the workouts execution should be considered a study limitation as participants may respond to the exercises according to the order they are presented rather than type of disciplines.

A physiological example of this order effect is that someone will feel much less stressed on the second or third exposure to experimental measurements, and therefore would respond differently, especially with regards to stress hormones, such as cortisol, even if the objectively measured heart rate response to workouts gives sufficient information about the intensity of each workout. This is very important, as literature well stated the positive relationships between exercise intensity and both cortisol and DHEA-S production (Giannopoulou et al., 2003; Copeland et al., 2002). According to our knowledge, this is the first study characterizing heart rate engagement of WWMT, a new fitness discipline, spread rapidly among fitness centres customers, in which even if the task is simply to walk it allows to reach high intensities of training for the high slope of treadmill (i.e. 15%) and the absence of motorized surface. Indeed, on a magnetic treadmill the real engine of the treadmill is the user through its gait, pushing the below surface. Our study also adds additional information to Luettgen and colleagues (2012) study, characterizing only mean intensity of Zumba® lesson.

Conclusions

The knowledge of acute and delayed hormonal effects of fitness disciplines is very important to optimize health promotion and maintenance of midlife women near to premenopause. Even if literature underlines the positive effects of classical walking workout (Morris et al., 1997), that is widely diffused and appreciated among women (Daley et al., 2011), our results underline the inability of its intensity to adequately stimulate LogDHEA-S production, in trained women and in the absence of slopes major than 3%, eliciting high LogCortisol to LogDHEA-S ratio, for several hours after workout conclusion. On the contrary, the intensity of both Zumba® and walking workout on magnetic treadmill has been shown able to elicit an important increase of LogDHEA-S production, and reduced LogCortisol to LogDHEA-S ratio, for several hours after workout conclusion. However, if the main objective of physical exercise practice is whole body health maintenance and health promotion, it is important to remind that classical walking workout is a low-impact discipline, meaning low musculoskeletal injuries risk and negative cardiovascular events risk, while both Zumba® and prolonged walking on a magnetic treadmill, at 15% of slope, are high-impact disciplines, meaning higher musculoskeletal injuries risk (Inouye et al., 2013; Tulchin et al., 2010) and hypothetical higher negative cardiovascular events risk, if participants have some risk factor.

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Authors' Qualifications

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