Activity of autonomic nervous system, energy expenditure and assessment of oxidative stress in menopause-women using hormone replacement therapy

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Abstract

Aims: Menopause is a period of significant physiological change that may be associated with increased body weight and obesity-related diseases. Many studies have been carried out to determine influences of estrogen depletion, resting

energy expenditure (REE) decline and aging during menopause-related obesity.

Methodology: In the present experiment, REE, body composition, activity of the autonomic nervous system, oxidative stress and food intake were measured in three groups of women: pre-menopause (n=40), post-menopause with hormone-replacement therapy (HRT; n=40) and post-menopause without HRT (n=40).

Results: In post-menopause women with HRT a significant increase was found in: 1) the sympathetic activity, measured by the power spectral analysis of the heart rate variability; 2) REE, measured by indirect calorimetry; 3) oxidative stress, measured by FRAS 4 compared to the value of the other two, while fat mass, measured by BIA, was reduced in favor of a recovery of free fat mass.

Conclusion: The study emphasizes the important changes due to HRT on various components influencing body weight in menopause-women.

Keywords: *Hormone-Replacement Therapy, Resting Energy Expenditure, Autonomic Nervous System, Body Composition, Assessment of Oxidative Stress.*

1. Introduction

Menopause is a period of significant physiological change that is largely related to estrogen depletion and subsequent cessation of ovarian function. During

the menopause period, women tend to gain weight and ~~FM (~~Fat Mass (FM) [1]. It is not clear whether the increase in adiposity is a consequence of the decline in endogenous estrogen. Several studies faced the question by using post-menopause Hormone-Replacement Therapy (HRT). If the increase in adiposity is a consequence of the decline in endogenous estrogen that occurs at this time, HRT should prevent or reduce body fat gain. However, existing clinical data addressing this issue are discordant. Anderson et al. [2] showed that short-term (2-month) use of HRT did not alter Body Mass Index (BMI), FM or Fat-Free Mass (FFM) in postmenopausal women [3]. With longer-term use (1 year), Reubinoff et al. [4] found a similar increase in body weight and FM among women taking HRT and those who declined its use. They did, however, observe that there was a significant shift from gynoid to android fat distribution only in women not taking HRT [5]. A decrease in body weight was found by Espeland et al. [6] over a 3-yr period in taking HRT women compared to no-taking HRT women. Conversely, other data suggested that oral estrogen might cause an increase in body fat, possibly by limiting lipid oxidation [7]. Thus, whether and how hormone therapy affects body composition in postmenopausal women is still unclear.

Ovarian hormones may influence body composition through several potential mechanisms. It has been suggested that estradiol inhibits the action of adipose tissue lipoprotein lipase, the enzyme that hydrolyzes circulating triglycerides, allowing for the uptake of fatty acids into adiposities [5]. Data from rodent models indicate that estrogen acts as an anorectic, decreasing voluntary food intake [8].

Furthermore, weight gain in postmenopausal women may depend on an accelerated Resting Energy Expenditure (REE) decline [9, 10]. In this regard, it was found that REE declines by approximately 420 kcal/day in post-menopause compared with premenopausal women [11].

REE accounts for 60–75% of total daily energy expenditure. REE decreases with age [12, 13]. The age-related decline in REE could be due not only to the loss of FFM and an alteration in its metabolically active components, but also to a

reduction in physical activity. It is well known that the reduction in physical activity leads to a reduction in REE and a~~an~~ decrease in FFM.

The decline in REE observed in postmenopausal women may depend on aging. However, REE seems to decrease more during the menopause transition than could be attributed to the aging process [14 ,15, 16]. During menopause transition, the decrease in REE accelerates the gains in FM which, in turn, may contribute to increasing the incidence of obesity-related diseases such as a worsening of cardiovascular risk profile [1] and Type II diabetes [14]. Also estrogen depletion by itself seems to increase cardiovascular risk [17–19]. Staessen et al. [17] observed that the incidence of hypertension was significantly higher in hypoestrogenic postmenopausal women when compared with women receiving HRT, after adjustment for age, race, and weight. Comparable findings were reported by Vongpatanasin et al. [18] and Weitz et al. [19]; they ~~in their studies,~~ conclud~~ed~~ed~~ing~~ that HRT lowered diastolic blood pressure in postmenopausal women. Regarding the metabolic variables evaluated, it was found that postmenopausal women not receiving HRT had significantly higher plasma cholesterol and TG levels than reproductive-age women, but, more importantly, the levels were also higher than in those receiving HRT [20].

Furthermore, oxidative stress occurs at menopause because of loss of estrogens, which have antioxidant effects on low-density lipoproteins [21]. Estrogens confer cardioprotection by lowering protein oxidation and antioxidant properties [22, 23]. Diminished antioxidant defense is associated with osteoporosis in post-menopause. Modulation of the estrogen receptors α and β has been reported to be effected in vitro by oxidative stress [24]. The atheroprotective effect of estrogen might also be partly due to its antioxidant action [25], resulting in a decrease of LDL oxidation [26]. In postmenopausal women, hormonal replacement therapy might either counteract the effect of a possible increased oxidative stress or improve antioxidant status.

Despite the key role of antioxidant micronutrients in preventing accelerated aging, data related to relationship between oxidative stress and antioxidant status

in menopausal women are scarce. Various factors contribute to the inter-individual variability in REE such as FFM [27], sympathetic nervous system (SNS) activity [28–32] and endocrine status (*e.g.* thyroid hormone).

The sympathetic nervous system (SNS) is an important control mechanism of the body. The SNS shows physiologic fluctuations with age which is considered to be related often to differences in the REE [33–35]. Heart rate variability (HRV) power spectral analysis is a well-accepted, useful, and non-invasive method, and has provided a comprehensive quantitative and qualitative evaluation of neuro-autonomic function under various research and clinical settings [36, 37, 38].

Previous investigations have demonstrated that the percentage of body fat [39], energy storage [40], and glucose-induced thermogenesis [41–43] are correlated with differences in the power spectral components. A series of recent studies with the HRV power spectral analysis have shown that obese young women possess significantly lower sympathetic activity against various thermogenic perturbations, such as cold exposure [44], capsaicin-containing yellow curry diet [45], and mixed food intake [46].

Although the relation between HRV and body mass index has been shown, as reported in the studies cited above, other authors have indicated that no correlation was noted between HRV and body mass index [29, 47]. On the other hand, Hirsch and Mackintos have reported their perplexity about the controversial influences of autonomic nervous activity (measured by HRV) on body weight [48].

The aim of this study was to determine whether healthy, obese menopausal women submitted to HRT treatment had changes of the REE, autonomic asset, and assessment of oxidative stress respect at obese pre and post menopausal women.

2. **Methods**

2.1 Participants

Sedentary female subjects (n=120) were enrolled among the subjects of the Clinical Dietetic Service of Second University of Naples, Italy. The subjects were divided into three groups: pre-menopause (n=40), post-menopause with hormone-replacement therapy (HRT) (n=40) and post-menopause without HRT (n=40).

Each subject had a normal physical examination and met the following inclusion criteria: non smoker, no medication or nutritional supplements that could influence metabolism or autonomic functions, with the exception of HRT, vitamins, and minerals. We defined menopausal status according to the definition of the Reproductive Aging Workshop (2011). At the time of the study, women had to either have been on HRT for at least 2 years or never have been on HRT. HRT treatment consisted of estrogen combined with progesterone (estrogen = 0.625 mg/day, progesterone = 2.5 mg/day).

Participants were provided with both written and oral information regarding the study protocol and were ensured that they were free to withdraw from the study at any time. All subjects gave their written informed consent before participation. All procedures conformed to the directives of the Declaration of Helsinki. The study was approved by the Human Ethical Review Committee of Second University of Naples. Furthermore, a medical examination ascertained the absence of any disease in each subject. Thus, the subjects utilized in this study were healthy and weight stable for a period of three months prior to the study. Age and anthropometric values, expressed as means ± SE, are reported in Table 1.

Table 1. Age, body mass index (BMI) and blood pressure (BP) in pre-menopause, post menopause and HRT women.

Table 1

Parameters	Pre-menopause (n=40)	Menopause (n=40)	HRT (n=40)
Age (years)	44.4±2.9	51.0±4.8	51.9±3.3
BMI (Kg/m²)	30.0±1.4	31.2±0.6	32.2±1.2
BP systolic/diastolic (mm Hg)	122.0±10.0/70.0±7.0	125.0±9.0/72.0±8.0	124.0±10.0/70.0±9.0

2.2. Indirect calorimetry

Resting energy expenditure (REE) was measured by indirect calorimetry using a computerized flow-through canopy-gas analyzer system (VMax 29, Sensor Medics, USA), which was calibrated with the precision gas mixture before each measurement. Samples of inspired and expired air were analyzed for the difference in oxygen and carbon dioxide concentrations through a paramagnetic differential oxygen sensor and an infrared carbon dioxide analyzer, respectively. Signals from the gas analyzers were processed by a computer-assisted software and oxygen consumption and carbon dioxide production were calculated once every minute for 30 min. The first 10 min was discarded and the mean value of the data for the remaining 20 min was used for calculations. REE (kcal/min) was calculated according to Ferrannini [49] and expressed as kcal/day. To describe the urea excretion during the calorimetry, urine was collected in a 12-hour interval, between 8:00 and 20:00. Urinary urea concentration was analyzed by a kinetic enzymatic method (Urea SYS 1, Boehringer Mannheim, Mannheim, Germany). The REE was adjusted by linear regression, according to Ravussin [14] for the variation of fat-free mass and age. The adjusted REE was calculated as the mean REE plus the individual measured REE value for each subject. The REE was measured in subjects after a 12 h period of overnight fasting. The measurements have been made between 8:00 A.M. and 11:00 A.M.

2.3. PSA of HRV

The PSA of HRV was evaluated by an electrocardiogram (ECG) for 5 min. The signals were acquired on a PC at 100 s/s by an electrocardiograph (delta-1 plus, Cardioline, Milan, Italy) connected to the serial port of a PC; a custom software made with LabView (National Instruments, Texas, USA) was used for data acquisition and analysis. The R waves were automatically recognized, and the R–R intervals were calculated and resampled to obtain a constant-time-based signal (100 ms). The Fourier transform was then applied to this signal and visualized in the form of power LF (0.04–0.15 Hz) and HF (0.15–0.40 Hz). The LF, HF and the LF/HF ratio were used to estimate the sympathetic and

parasympathetic activities. Although the time window for HRV recording is generally greater than 5 min, the Task Force on HRV [38] indicates that main spectral components are distinguished in a spectrum calculated from short-term recordings of 2 to 5 min.

2.4. Body composition

Body composition was determined by conventional Body Impedance Analysis (BIA) with a single-frequency (50 kHz) bioelectrical impedance analyzer (BIA 101 RJL, Akern Bioresearch, Firenze, Italy), according to the standard tetrapolar technique, with the subject in supine position and the electrodes placed on the dorsal surface of right foot and ankle, and right wrist and hand [50]. Patients were asked to refrain from strenuous exercise and to maintain their usual intake of caffeinated beverages during the 3 days preceding the measurements. After overnight fasting, patients were invited to empty the bladder before being evaluated. Body composition was then calculated by bioelectrical measurements and anthropometric data using the software provided by the manufacturer, which incorporated validated predictive equations for total body water, fat mass and fat free mass (FFM). All the participants to the study were submitted to the BIA between the eighth and eleventh day from the onset of the menstrual cycle. They had been fasting for 12 h, they had not assumed (consumed) drinks for 4 h and they had not assumed (consumed) contraceptive(s) over the last three months; this condition assured an optimal state of hydration for BIA [44]. For reasons of brevity, only the percentage of FFM (calculated as kg of FFM/kg of total body weight) has been reported.

2.5. Free Radical Analytical System 4 (Fras-4)

Total ROS production was measured using Free Radical Analytical System 4 kits (d-ROMs test kit; Diacron, Grosseto, Italy). ROS and ROS derivatives react with a suitably buffered chromagen, yielding a colored compound that is measured photometrically at a maximum absorbency peak of

505 nm. The value is directly proportional to the concentration according to the Lambert-Beer law. The degree of ROS production was expressed as Carr Units as established by the manufacturer.

2.6. Other parameters

Blood pressure was measured by the Riva–Rocci method in the sitting position after a 5-minute rest using a mercury sphygmomanometer. An average of two measurements wasere used as representative of the patient's blood pressure status (Table 1). Blood tests showed normal values for cholesterol, triglycerides, azotemia, and thyroid hormones.

2.7. Statistical analysis

Data was analyzed using the GraphPad Prism 6 software for Windows (Microsoft, USA). The analysis of variance for repeated measures (ANOVA) was used to determine differences among the dependent variables for the effect of training stage and subjects' age. When indicated by a significant F value, a post hoc test using the Bonferroni multiple comparisons was performed to identify significant differences between groups. Multivariate regression analysis was performed in order to evaluate the role of confounding factors on results. All data were reported as means ± SE. Statistical significance was considered for $p \leq 0.05$.

3. Results

Figure 1 shows that the REE of HRT women is higher than of pre-menopause and,—post-menopause women. The analysis of variance showed significant effect [$F(2, 117) = 18.4$, $p<0.01$]. Post hoc test showed a difference between HRT and pre-menopause, post-menopause women.

Figure 2 shows that the percentage of FFM of HRT is higher than of pre-menopause and post-menopause women. The analysis of variance showed significant effect [$F(2, 117) = 3.14$, $p<0.01$]. Post hoc test showed a difference between HRT and pre-menopause or, post-menopause women.

Figure 3 reports the values of LF in HRT are higher than of pre-menopause and post-menopause women. The analysis of variance showed

significant effect [F (2,117) = 7.59, p<0.01]. Post hoc test showed a difference between HRT and pre-menopause or~~,~~ post-menopause women.

Figure 4 d reports the values of HF. HF values of pre-menopause and~~,~~ post-menopause women are similar ~~than~~to that ~~values~~ of HRT women. The analysis of variance showed no significant effect [F (2, 117) = 12.23, p=0.16].

Figure 5 reports the values of d-ROMs test. ~~in~~ HRT value is~~are~~ higher than of pre-menopause and post-menopause women. The analysis of variance showed significant effect [F (2,117) = 2.39, p<0.01]. Post hoc test showed a difference between HRT and pre-menopause or~~,~~ post-menopause women.

Multivariate regression analysis showed that there were no significant correlations between the variables examined.

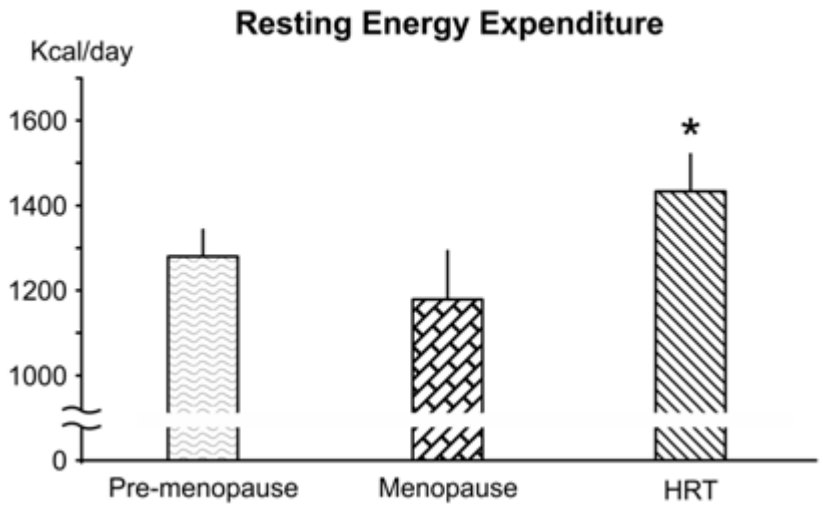

Figure 1. Changes in resting energy expenditure in pre-menopause, post menopause and HRT women. The asterisk indicates a statistical significant difference compared to other groups ($p < 0.01$).

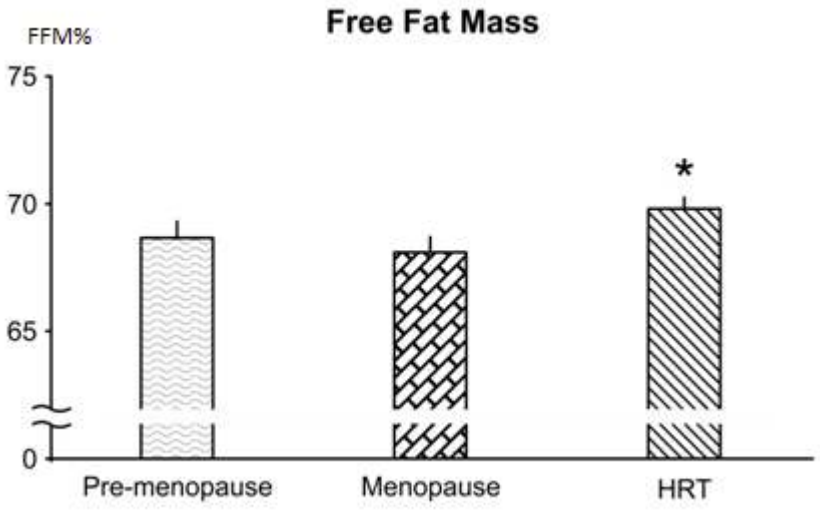

Figure 2. Changes in free fat mass (FFM) in pre-menopause, post menopause and HRT women. The asterisk indicates a statistical significant difference compared to other groups ($p < 0.01$).

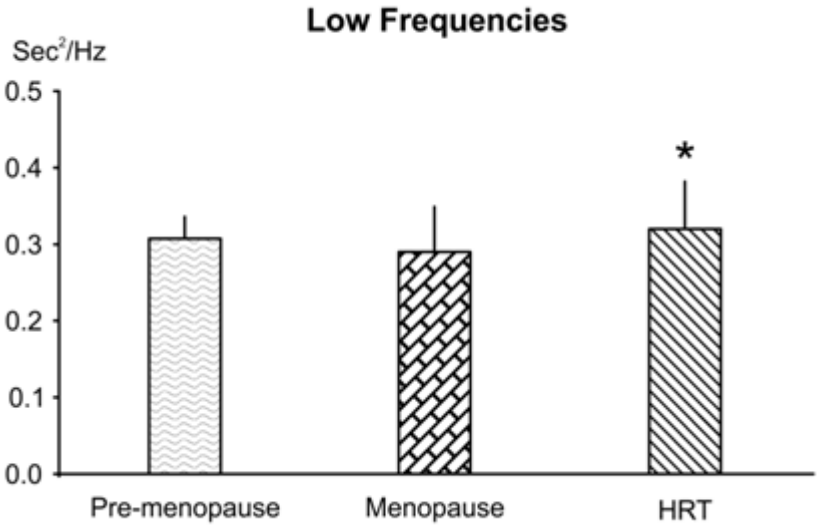

Figure 3. Changes in Low frequencies of heart rate variability in pre-menopause, post menopause and HRT women. The asterisk indicates a statistical significant difference compared to other groups ($p < 0.01$).

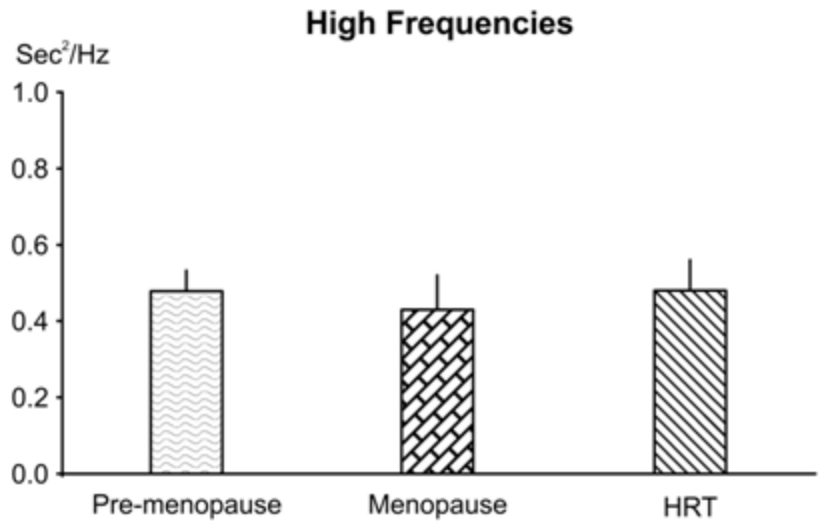

Figure 4. Changes in high frequencies of heart rate variability in pre-menopause, post menopause and HRT women.

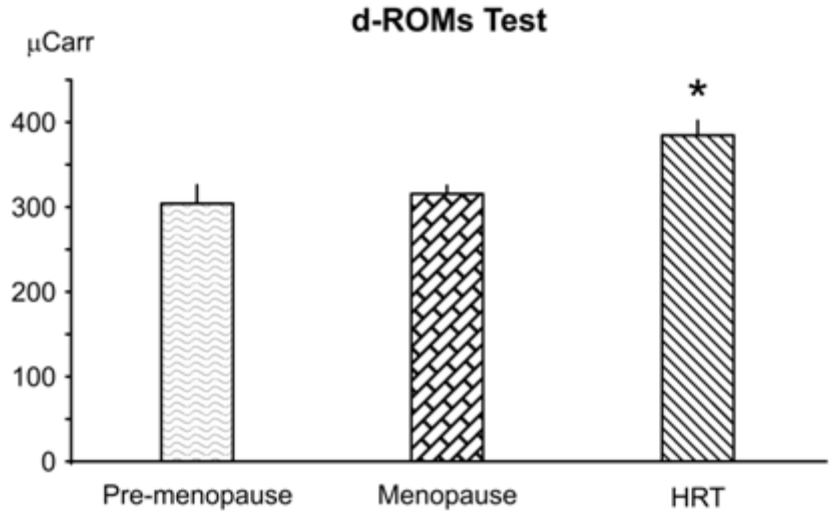

Figure 5. Changes in in d.ROMs test in pre-menopause, post menopause and HRT women. The asterisk indicates a statistical significant difference compared to other groups ($p < 0.01$).

4. Discussion

This study reports for the first time the difference in REE, sympathetic function and oxidative stress between women in pre menopause, menopause or under HRT. The present experiment indicates a modification of vegetative modulation in HRT women and the increase of autonomic control regarding the

sympathetic component. The increase of the sympathetic branch is an important factor in maintaining the highest REE in women in HRT compared to pre-menopausal and menopausal women. In this experiment, the autonomic activity of pre-menopausal and menopausal women is lower than that of HRT subjects. The same reduction of vegetative control was found in all groups, so that there was no difference.

The age-related decline in REE is due to alteration in metabolically active components, as metabolic changes induced by menopause. Indeed, suppression of sex hormones to post-menopausal levels reduces REE in young healthy women, probably through a reduction of sympathetic activity [33, 36, 51–53]. For the present experiment, a possible explanation for the lack of REE- and FFM-decline in obese women could be that the activity of the sympathetic nervous system does not decrease during the aging, in spite of the changes in the level of sex hormones. The sympathetic nervous system is involved in the control of body weight, partly through its effect on energy expenditure [54]. This elevated sympathetic activity could also explain the lack of FFM-decline, because the trophism of skeletal muscle (very important component of FFM) is positively affected by the sympathetic discharge [55].

The originality of the present experiment is to emphasize the difference sympathetic activity induced by HRT and then on the relationship between the sympathetic nervous system and REE. It has demonstrated a significant influence of sympathetic activity on eating behavior, also through an increase in thermogenesis [56].

The results of the present experiment are consistent with the hypothesis that a reduction in autonomic activity could play a determinant role in the increase in food intake and in the induction weight gain in menopausal women [57].

This experiment, on the one hand, emphasizes aspects regarding the complex relationship between the autonomic nervous system and body weight in HRT and menopause. Therefore, these findings could be useful in the elucidation of pathologic mechanisms related to obesity and aging in women.

On the other hand, the role of estrogen as antioxidant in vivo is a matter of debate [58–61]. Controversies still exist regarding the beneficial protecting effect of HRT. In some trials estrogen replacement therapy had a beneficial effect in prevention of coronary artery disease, morbidity and mortality [62]. However, pooled data from clinical trials does not support the notion that HRT prevents cardiovascular events [63]. Moreover, the only randomized data available to date does not support any beneficial effect in postmenopausal women with coronary heart disease [64].

There are several mechanisms by which estrogen exposure may increase breast cancer risk, such as increasing cell proliferation and opportunities for random errors during DNA replication [65]. However, estrogen metabolism also generates reactive oxidative species [66, 67]. Whereas modest levels of reactive oxidative species are necessary for cell signaling processes [68], excess reactive oxidative species can damage DNA, lipids, and proteins [69]. The metabolism of estrogens results in the generation of reactive quinones capable of forming adducts with DNA and of participating in redox cycling, thereby generating additional reactive oxygen species [70]. Consequently, estrogen metabolites have the ability to directly and indirectly result in oxidative damage to cellular components as well as to disrupt signaling processes such as those required for cell growth or apoptosis [68].

HRT preparations often contain equine estrogen; metabolites of some equine estrogens possess greater potential for causing oxidative damage than that caused by human estrogens [71]. A metabolite of equine estrogen, 4-hydroxyequilenin, has been shown to cause oxidative damage and single-strand breaks in λ phage DNA [72] and in breast cancer cell lines, especially ER-positive cell lines [73].

The findings of the present study agree with other literature data. In fact, it has been well documented that menopause is associated with a decrease of resting energy expenditure [74].

Another Metabolic studies have found that soy, which contains isoflavones, exerts a lipid-lowering effect, favours vasodilatation and arterial compliance and contributes to regulate fasting glucose and insulin levels in humans. In addition, phytoestrogens by their estrogenic properties may favourably affect muscle mass. Nevertheless, it is unknown if isoflavone supplementation could increase FFM. One explanation about this effect could be that skeletal muscle is an important site of estrogen receptors α (ER α) and β (ER β) and that phytoestrogens are known to have estrogenic properties [75]. In this sense, it has been demonstrated previously that soy protein supplementation has an effect on hip lean mass in perimenopausal women 40g/day for 24weeks; and on lean body mass in elite athletes, 1.5 g/kg/day for 8 weeks.

5. Conclusion

In conclusion the effect of HRT remains controversial. In untreated postmenopausal women, optimal antioxidant micronutrient intakes could be a powerful tool in counteracting the effect of hormonal modifications in terms of oxidative stress.

This type of research analyzed aging effects, as in part shown in previous research on fertile women and on subjects in menopause [35, 36], aging modifies the type of adaptation of oxygen waste and of the autonomic nervous system activity.

Under these conditions, nutrition could offer an interesting alternative way in preventing aging. Finally, practical implications of energetic and autonomic adaptations shown in this study can include different strategies for prevention and therapy of obesity, a pandemic disease in the Western World.

Limitations

The major limitations of the present study are in the techniques used for the evaluation of body composition and cardiac vegetative regulation. In fact, both BIA and HRV-PSA cannot give a precise quantification of body composition and

vegetative output, respectively, but should be considered semi-quantitative indexes of this factors; nevertheless, at date there is no better non-invasive alternative to these techniques.

Conflict of interest

The authors declare that they have no competing interests.

Author's contribution

Antonietta Messina carried out biological assays and with the contribution of Anna Valenzano, Fiorenzo Moscatelli carried out the patient evaluations. Vincenzo De Luca, Andrea Viggiano, Domenico Tafuri, participated in the design of the study. Antonio Ivano Triggiani, Fiorenzo Moscatelli, performed the statistical analysis. Marcellino Monda, Giovanni Messina, Sergio Chieffi and Giuseppe Cibelli, conceived of the study, and participated in its design and coordination and helped to draft the manuscript. All authors read and approved the final manuscript.

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