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Relationship between physical activity and markers of oxidative stress in independent community-living elderly individuals

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ABSTRACT

The aim of the present study was to examine the relationship between objective data of physical activity and markers of oxidative stress in older men and women. Participants were old adults, aged ≥ 60 years (61 women and 34 men) who were all capable of performing basic daily activities by themselves and lived on their own. To describe physical activity we used objective data measured by accelerometers which record active and sedentary periods during everyday life for five days. Determination of oxidative stress was conducted from three perspectives: determination plasma total antioxidant status (TAS), plasma antioxidant enzyme activities, i.e., glutathione peroxidase (GPx), catalase (CAT) and superoxide dismutase (SOD), and membrane lipid peroxidation (TBARS). In the group of women, those who met physical activity recommendations (WR) had lower level of TAS. In addition, the moderate to vigorous physical activity (MVPA) was negatively correlated with TAS. Simultaneously, MVPA was correlated with increase in the GPx antioxidant enzyme activity, and the counts per minute were positively correlated with CAT activity. In the group of men, the cpm and the MVPA were negatively correlated with lipid peroxidation while lifestyle physical activity was positively correlated with CAT activity.

These findings suggest that MVPA in the elderly although it is related to a decrease in the TAS in women, induces adaptive increase in antioxidant enzyme activity and decreases lipid peroxidation in both women and men. These results suggest that at this time of life, it is not only the amount of physical activity performed that is important but also its intensity.

1. Introduction

Evidence has shown that physical activity is a key factor in achieving a healthy aging (Buchner, 2009). The physical activity is associated with favorable changes in blood pressure, lipid and lipoprotein profiles, markers of inflammation, with reductions in the risks of cardiovascular disease, type II diabetes, depression, cancer, all-cause mortality even with a better perceived health (Lohne Seiler et al., 2014; O'Donovan et al., 2010). On the other hand, physical inactivity is associated with a variety of diseases and pathologies such as cardiovascular disease, type II diabetes, muscle atrophy, obesity and even Alzheimer and Parkinson (Coelho et al., 2014; Jiang et al., 2013; Loprinzi et al., 2013; Ranasinghe et al., 2014). In addition, recent research shows that physical activity contributes significantly to a longer life in good health (May et al., 2015).

Despite its many benefits, physical activity increases maximal oxygen consumption (VO_2 max), and thus resulting in increased generation of Reactive Oxygen Species (ROS), which have a high oxidizing capacity (Ji et al., 2006; Powers et al., 1999; Takahashi et al., 2013a). ROS are essential signaling molecules involved in fundamental biological processes at adequate levels, such as muscle contraction and subsequent fatigue, which are important mechanisms to prevent cell damage after performing physical activity (Radak et al., 2013). The ROS are also involved in mechanisms such as satiety, adipocyte differentiation and insulin release. However, an excess of ROS can cause oxidative damage (Al-Mehdi et al., 2012; Mailloux et al., 2013; Woolley et al., 2013). Several researchers have reported that, even though physical activity increases ROS generation, it is also able to activate adaptive responses that induce gene expression of antioxidant defense systems (Ji et al., 2006; Palasuwan et al., 2011; Powers et al., 1999; Radak et al., 2005, 2008; Rowiński et al., 2013). However, during the aging,

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adaptability of the organism gradually diminishes and this increases susceptibility to oxidative stress (Bailey et al., 2010; Harman, 1956; Ji, 2001). The causes may be increased free radical production and/or decreased levels of antioxidants in the body (Nikolaidis et al., 2008).

To the knowledge of the authors, there is no definitive evidence in the relationship between the intensity of physical activity and markers of oxidative stress in older adults. Therefore, it is necessary to observe how the body of the elderly behaves with physical activity and if it is able to implement compensatory mechanisms such as the stimulation of antioxidant enzymes, that control the levels of ROS released.

The aim of the present study was to examine the relationship between objective data of physical activity and markers of oxidative stress in older men and women.

2. Material and methods

2.1. Sampling and recruitment

The present study included 95 participants (61 women and 34 men aged 60–85) who were able to perform basic activities of daily life independently and lived in their own home. The inclusion criteria were: old adults, aged ≥ 60 years who were all capable of performing basic daily activities by themselves and lived on their own. The exclusion criteria were: individuals with cognitive impairment, with a dementia diagnosis or mentally disabled. They were recruited in our collaborating senior centers situated in San Sebastian ("Gure Egunsentia" senior center and "Aulas de la Experiencia" college for adults) and Zarautz ("Udaberri" senior center) in Spain. These centers were chosen on the basis of the willingness to collaborate of their members. All participants gave written informed consent and the study was approved by the Ethics Committee of the "Matia Instituto Gerontológico" Foundation.

2.2. Anthropometric measurement

Height and weight were measured using height boards and weighing scales (Añó Sayol SL, Barcelona, Spain). The weight (kg) and height (cm) of the participants were then used to calculate the body mass index (BMI in kg/m^2). All measurements were taken by the same person following the standards of the International Society for the Advancement of Kinanthropometry (ISAK, 2001).

2.3. Blood pressure measurement

Blood pressure (BP) was measured in the right arm while the participant was in a seated position with an automatic sphygmomanometer (Omron M5-1, OMRON Corporation, Japan). All measurements were taken by the same person following a validated protocol (Panamerican Hypertension Initiative, 2003).

2.4. Measurement of physical activity

To be able to accurately measure physical activity we used accelerometers that record active and sedentary periods during everyday life. Accelerometers offer a number of desirable features for monitoring human movement: amount, duration, frequency and intensity (Mathie et al., 2004). In this study, accelerometry was performed using the Actigraph GT3X model (Actigraph LLC, Pensacola, FL, USA). The monitor is worn on the hip with a belt (Garatachea et al., 2010) and participants were asked to wear one for a 5 day period removing it only for sleeping and bathing. All the participants wore the accelerometers from Tuesday to Saturday to take into account also, at least, the activity done during one of the days of the weekend.

Activity was recorded using 10-second epochs. Data files recorded on the accelerometers were downloaded and processed with Actilife software (version 5, Actigraph, 2011). For the analysis presented here, only days on which the monitors were worn for 10 or more hours were considered valid and it was required that there were at least three days of data to validate the recorded data (Hart et al., 2011). Non-wear time was defined by an interval of at least 60 consecutive minutes of zero activity intensity counts, with tolerance for 2 min of counts between 0 and 50. The summary variables presented in this analysis were as follows: number of steps per day (spd), mean counts per minute (cpm) and number of minutes per day spent in intensity-specific categories. It is difficult to decide the cut-off points to classify the intensity of physical activity in older people because there is currently no consensus in the scientific literature. In the present study the cut-off points to classify the intensity of physical activity follow the classification developed by Freedson et al. (1998), where the cut-off point for moderate activity was set in 1952 cpm. To classify lower intensity activities we took as reference the article by Lohne Seiler et al. (2014). Thus, the following intensity-specific cut-off points were applied to the raw data; sedentary time was defined as all activity below 100 cpm (Lohne Seiler et al., 2014), low-intensity physical activity was defined as all activity between 100 and 759 cpm (Lohne Seiler et al., 2014), time in lifestyle activity was defined as all activity between 760 and 1951 cpm and moderate to vigorous physical activity (MVPA) was defined as all activity ≥1952 cpm (Freedson et al., 1998). The number of minutes per day at different intensities was determined by summing all minutes where the count met the criterion for the specific intensity, divided by the number of valid days.

For a first analysis, the participants were divided into groups according to sex and level of physical activity. As our sample was very active this classification was based on recommendation of daily step goals of 10,000 steps for healthy adults (Choi et al., 2007; Tudor-Locke et al., 2011). So, we obtained the following subgroups: women who did not meet physical activity recommendations (WNR), women who met physical activity recommendations (WR), men who did not meet physical activity recommendations (MNR) and men who met physical activity recommendations (MR).

Table 1
Anthropometric characteristics, blood pressure, physical activity data and markers of oxidative stress of subjects in the study (mean ± SD).

Variables[a]	Women (n = 61)	Men (n = 34)
Anthropometric characteristics		
Age (years)	70.1 ± 6.6	70.6 ± 7.4
BMI (kg/m^2)	27.3 ± 3.7	27.5 ± 2.8
Waist perimeter (cm)	87.7 ± 9.1 [A]	98.1 ± 9.1
Waist–hip index	0.84 ± 0.06 [A]	0.94 ± 0.06
Blood pressure		
Systolic BP (mm Hg)	145 ± 18.5 [A]	156 ± 21.9
Diastolic BP (mm Hg)	77.5 ± 9.3	81.8 ± 11.4
Variables of physical activity		
Counts per minute (cpm)	262 ± 117	262 ± 107
Steps per day	11850 ± 4574	11743 ± 4148
Sedentary PA (min/day)	521 ± 65.5	526 ± 71.6
Low-intensity PA (min/day)	136 ± 36.6 [A]	114 ± 29.6
Lifestyle PA (min/day)	70.2 ± 21.0	61.9 ± 26.0
MVPA (min/day)	72.8 ± 39.2	75.9 ± 40.7
Variables of oxidative stress		
TAS (mmol/l)	1.09 ± 0.25 [A]	1.33 ± 0.33
GPx (nmol/min/ml)	111 ± 31.7 (n = 55)	107 ± 31.7 (n = 30)
CAT (nmol/min/ml)	15.2 ± 11.2	15.2 ± 13.7
SOD (U/ml)	5.56 ± 4.67 (n = 44)	5.12 ± 3.41 (n = 24)
TBARS (μM MDA)	16.6 ± 11.8 (n = 38)	13.1 ± 13.0 (n = 20)

[a] BMI, body mass index; BP, blood pressure; PA, physical activity; MVPA, moderate to vigorous physical activity; TAS, total antioxidant status; GPx, glutathione peroxidase activity; CAT, catalase activity; SOD, superoxide dismutase activity; TBARS, thiobarbituric acid reactive substances.

[A] Significantly different from the mean value (unpaired Student's *t* test, $p < 0.05$).

Table 2
Characteristics of subjects in the study according to their physical activity (mean ± SD).

Variables[a]	Group[b]			
	WNR (n = 25)	WR (n = 36)	MNR (n = 12)	MR (n = 22)
Age (years)	73.3 ± 6.26[A]	67.3 ± 5.59	74.0 ± 7.22	69.2 ± 6.95
BMI (kg/m^2)	27.7 ± 3.71	26.6 ± 3.42	28.7 ± 3.12	27.0 ± 2.43
Waist perimeter (cm)	90.3 ± 7.61[B]	85.3 ± 9.78	103 ± 8.46[C]	95.8 ± 8.34
Waist–hip index	0.85 ± 0.05	0.83 ± 0.06	0.97 ± 0.05[C]	0.93 ± 0.05

[a] See Table 1.
[b] WNR, women who did not meet physical activity recommendations; WR, women who met physical activity recommendations; MNR, men who did not meet physical activity recommendations; MR, men who met physical activity recommendations.
[A] Significantly higher compared to WR group ($p < 0.001$).
[B] Significantly higher compared to WR group ($p < 0.05$).
[C] Significantly higher compared to MR group ($p < 0.05$).

2.5. Analytical procedures

Blood samples for oxidative stress determination were obtained after a minimum of 8 h fasting with the subject in sitting position into heparinized tubes with lithium heparin. The blood was immediately centrifuged at 3000 rpm during 10 min (Centrifuge BioTek Synergy HT). Plasma was separated into several aliquots and rapidly frozen at −80 °C until its analysis (Ultrafreezer −86 °C Sanyo vip Series MDF-U53V).

2.5.1. Total antioxidant status

Total antioxidant status (TAS) was measured with a kit (NX2332) from Randox Laboratories. Briefly, this TAS assay relies on the ability of antioxidants present in plasma to inhibit the oxidation of ABTS (2,20-azino-bis (3-ethylbenzthiazoline-6-sulphonicacid)), and this is assessed by reading the absorbance at 600 nm (Re et al., 1999).

2.5.2. Antioxidant enzyme activities

Antioxidant enzyme activities were measured in plasma with Cayman Chemical Company assay kits (Cayman Chemical, USA). Activity of glutathione peroxidase (GPx) was measured using an assay kit from Cayman Chemical (703102). This kit can be used to measure all of the glutathione-dependent peroxidases in plasma, as GPx activity is indirectly measured by a coupled reaction with glutathione reductase (GR). Specifically, oxidized glutathione (GSSG) produced by GPx is recycled to its reduced state by GR and NADPH. The $NADP^+$ is accompanied by a decrease in absorbance at 340 nm (Paglia and Valentine, 1967). The activity of GPx was expressed in nmol/min/ml. One unit was defined as the amount of enzyme that caused the oxidation of 1.0 nmol of NADPH to $NADP^+$ per minute at 25 °C. The catalase activity (CAT) was measured using a Catalase assay kit from Cayman Chemical (707002). This kit uses one of the two activities of the enzyme, the peroxidatic activity, in which methanol can serve as electron donor in the presence of an optimal concentration of H_2O_2. The formaldehyde produced was measured colorimetrically with peak absorbance at 540 nm (Johansson and Borg, 1988; Wheeler et al., 1990). The activity of CAT was expressed in nmol/min/ml. One unit was defined as the amount of enzyme that caused the formation of 1.0 nmol of formaldehyde per minute at 25 °C. Activity of superoxide dismutase (SOD) was measured photometrically using a Superoxide Dismutase assay kit (706002) also from Cayman Chemical. This assay is based on the competition between SOD and tetrazolium salt for detection of superoxide radicals generated by xanthine oxidase and hypoxanthine (Nebot et al., 1993). One unit of SOD was defined as the amount of enzyme needed to exhibit 50% dismutation of the superoxide radical and was expressed in U/ml.

2.5.3. Lipid peroxidation

Lipid peroxidation is a well-established mechanism of cellular injury in both plants and animals and is used as an indicator of oxidative stress in cells and tissues. The measurement of thiobarbituric acid reactive substances (TBARS) was based on a well-established method for screening and monitoring lipid peroxidation (Armstrong and Browne, 1994; Yagi, 1998). This was performed using the TBARS assay kit (10009055) again from Cayman Chemical in which the MDA–TBA adduct formed by the reaction of malondialdehyde (MDA) and thiobarbituric acid (TBA) under high temperature (90–100 °C) and acidic conditions is measured colorimetrically at 530–540 nm. Lipid peroxidation was expressed in μM MDA.

2.6. Statistical analysis

All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS Inc., version 21). In the group of women (n = 61) normality test was performed (Kolmogorov–Smirnov). Analyzed variables met the criteria of normality except age, CAT, SOD and TBARS. In the group of men (n = 34) normality test were performed (Shapiro–Wilk). In this case, analyzed variables met the criteria of normality except age, GPx and CAT. Depending on the normality of the dependent variable, the differences between groups were compared using the Student's *t*-test or U Mann–Whitney respectively and the data are presented as mean ± standard deviation (SD). Pearson's partial correlation analysis, with age as the control variable was carried out to ascertain the inter-relationship between the intensity of physical activity and markers of oxidative stress. The results presented as Pearson's correlation coefficient (r). The differences were considered to be statistically significant with $p < 0.05$.

3. Results

Ninety five subjects aged ≥ 60 years with mean age 70.3 and a standard deviation of 6.85 years were enrolled in the study. Anthropometric characteristics, blood pressure, physical activity data and markers of oxidative stress are shown in Table 1.

As shown in Table 2, age was higher in the groups where physical activity recommendations were not met, this difference being statistically

Table 3
Markers of oxidative stress of subjects in the study according to their physical activity (mean ± SD).

Variables[a]	Group[b]			
	WNR (n = 25)	WR (n = 36)	MNR (n = 12)	MR (n = 22)
TAS (mmol/l)	1.19 ± 0.23[A]	1.00 ± 0.22	1.35 ± 0.42	1.29 ± 0.28
GPx (nmol/min/ml)	106 ± 38.9 (n = 20)	116 ± 27.1 (n = 35)	98.5 ± 22.0 (n = 10)	111 ± 35.4 (n = 20)
CAT (nmol/min/ml)	11.5 ± 9.05[B]	17.2 ± 10.8	18.1 ± 20.3	14.3 ± 8.50
SOD (U/ml)	5.28 ± 5.69 (n = 19)	5.73 ± 4.23 (n = 25)	5.11 ± 3.65 (n = 7)	5.12 ± 3.43 (n = 17)
TBARS (μM MDA)	19.1 ± 13.4 (n = 17)	15.0 ± 9.88 (n = 21)	19.5 ± 17.6 (n = 7)	10.5 ± 9.13 (n = 13)

[a] See Table 1.
[b] See Table 2.
[A] Significantly higher compared to WR group ($p < 0.005$).
[B] Significantly lower compared to WR group ($p < 0.05$).

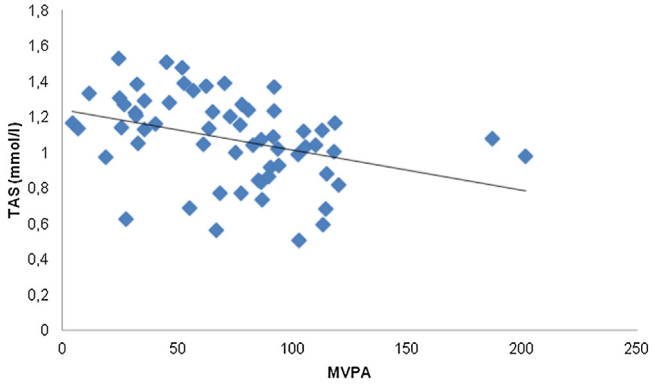

Fig. 1. Relationship between moderate to vigorous physical activity (MVPA) and total antioxidant status (TAS) in the group of women ($r = -0.318$; $p < 0.05$).

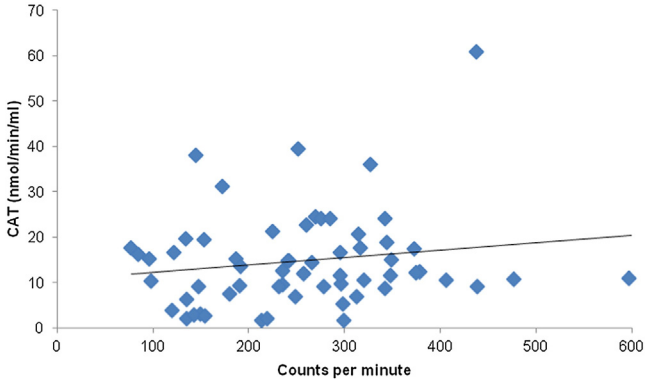

Fig. 3. Relationship between counts per minute and catalase activity (CAT) in the group of women ($r = 0.258$; $p < 0.05$).

significant only for women. There were no significant differences in BMI between examined groups although there was a tendency to lower BMI in groups who met physical activity recommendations. Waist perimeter was significantly lower in groups of women and men who met recommendations. The waist–hip index was lower in the more active groups but it only showed statistical significance in the group of men.

As shown in Table 3, TAS was significantly lower in the WR group than in the WNR group. The activities of antioxidant GPx and SOD were similar in both groups of women and men. Nevertheless the CAT activity was significantly higher in the WR group than in the WNR group. In the groups of WR and MR there was a tendency to lower lipid peroxidation, but this difference did not reach statistical significance.

In the group of women, TAS correlated negatively and significantly with the time spent on MVPA (Fig. 1). We also observed a positive and significant relationship between the time spent on MVPA and GPx activity (Fig. 2) as well as a positive relationship between CPM and CAT enzyme activity (Fig. 3).

In the group of men, lifestyle physical activity was correlated positively with CAT activity (Fig. 4). Moreover, more intense physical activity, expressed in counts per minute, and the MVPA were negatively correlated with TBARS (Figs. 5, 6), an indicator of damage in cellular lipid membrane. This indicates that the longer the time spent on intense physical activity, the lower is the damage in the membranes.

4. Discussion

In our study we observed that accelerometer-determined physical activity decreases in the elderly as described in other studies using the same methodology (Davis et al., 2011; Lohne Seiler et al., 2014). Although we did not find significantly lower values of BMI in the most active groups, physically more active men and women had lower anthropometric parameters suggestive of cardiovascular risk: waist circumference and waist–hip index. Other authors also observed lower anthropometric parameters as well as a reduction in cardiovascular risk associated with the physical activity in the elderly (Batty, 2002; Gregg et al., 2003; Harris et al., 2009).

During aging, the body gradually decreases its adaptability and increases susceptibility to oxidative stress (Bailey et al., 2010; Harman, 1956; Ji, 2001). In the present study, both in women and men, the TAS was lower in the more active groups, although statistical difference only appeared between WR and WNR groups. We also observed a significant and negative correlation between TAS and time on MVPA. This may be related to an increase in ROS generation as a result of more intense activities. Several studies in young people associated increased activity with TAS immediately after the activity (Ascensão et al., 2008; Gravina et al., 2011; Morales-Alamo and Calbet, 2014). However, other studies report an increase in markers of lipid peroxidation and a decrease in TAS after intense physical exercise, concluding that such an intense exercise induces a state of cellular damage and oxidative stress (Teixeira et al., 2013). For this reason, special precautions are recommended to reduce the risk of injury in athletes (Lamberti et al., 2013). This could corroborate that physical activity follows a curve of hormesis, as is suggested by several studies (Ji et al., 2006; Radak et al., 2008; Stranahan and Mattson, 2010). Although we did not determine the TAS directly after physical activity, we observed a decrease in this parameter in the group of women.

In the present study we observed that in the group of women time spent on MVPA positively correlated with the GPx activity while the increase in CPM was associated with CAT activity. In the group of men time spent on lifestyle physical activity correlated with CAT activity. We found that most significant correlations appeared in the female group between physical activity and antioxidant enzyme activity. We

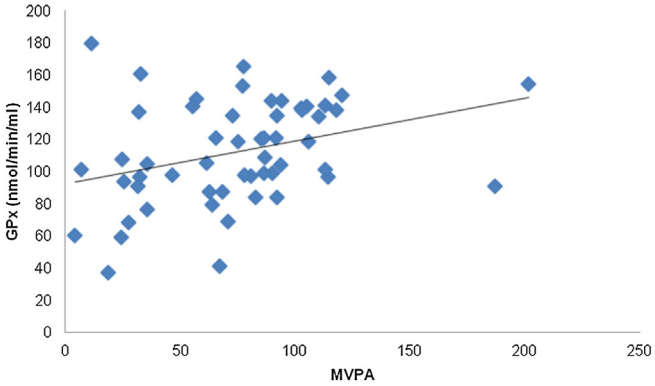

Fig. 2. Relationship between moderate to vigorous physical activity (MVPA) and glutathione peroxidase activity (GPx) in the group of women ($r = 0.273$; $p < 0.05$).

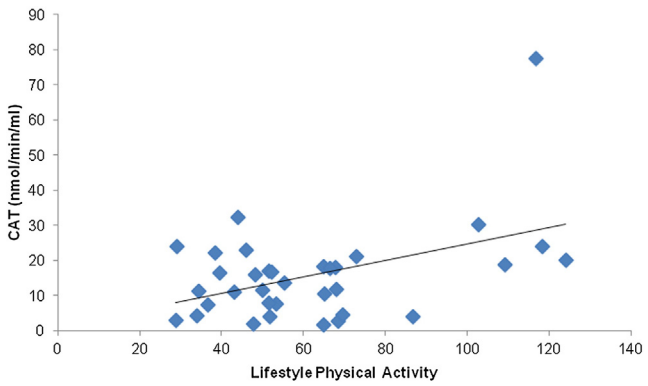

Fig. 4. Relationship between lifestyle physical activity and catalase activity (CAT) in the group of men ($r = 0.410$; $p < 0.05$).

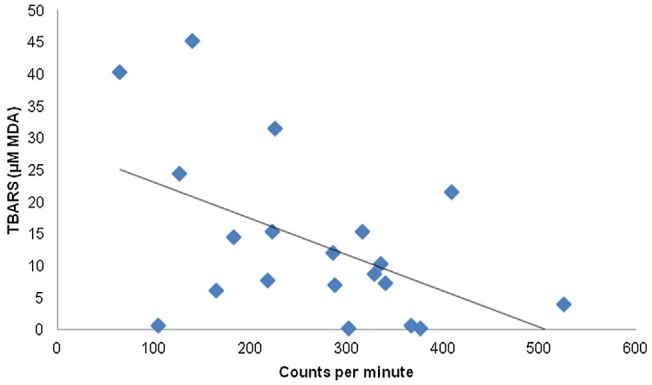

Fig. 5. Relationship between counts per minute and thiobarbituric acid reactive substances (TBARS) in the group of men ($r = -0.519$; $p < 0.05$).

also note that in the group of women the MVPA was the parameter related to CAT activity increase, while in the male group were less intense activities such as those carried out in daily life, which were more strongly associated to the antioxidant system.

Studies in older people also observed an increase in antioxidant activity associated with increased physical activity, although the antioxidant markers that they found were not exactly the same as we have found in the present study. Rowiński et al. (2013) concluded that "physical activity can reduce oxidative stress markers and induce adaptive increase in the antioxidant enzyme activity even in older men and women" (Rowiński et al., 2013), but the enzyme activity they observed was SOD. In any case, we must note that in this study physical activity was determined subjectively through questionnaires. Moreover, Takahashi et al. (2013a,b), in a study conducted in 29 older adults, determined the physical activity by accelerometry and also observed that different markers of antioxidant capacity correlated positively with the amount of physical activity in women. In the same study, SOD activity was positively correlated with the amount of physical activity in men (Takahashi et al., 2013a). In another article, Takahashi et al. (2013a,b) described an increase in GPx activity and a decrease in CAT activity in older adults following an intervention by low-volume exercise training (Takahashi et al., 2013b). Also Rosado Pérez et al. (2013) observed a significant increase in SOD and GPx activity in a group of Mexican older adults after the regular performance of moderate physical exercise (Rosado Pérez et al., 2013). As it is suggested by several investigations, this may be because a compensatory balance exists among various components contributing to the overall antioxidant defense system. As a consequence, the organism can compensate the decrease in a particular antioxidant by an increase in another one (K^{3}apciñska et al., 2000; Mecocci et al., 2000). Our results support the conclusions of Traustadottir et al. (2012) that demonstrated that in older adults, greater physical fitness is associated with greater capacity to resist an oxidative challenge, being the role of antioxidant enzymes critical (Traustadottir et al., 2012).

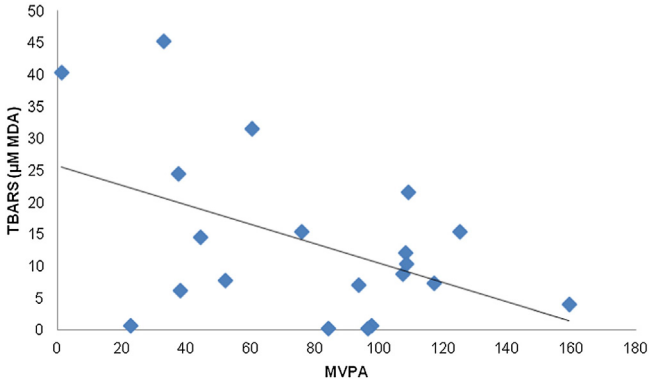

Fig. 6. Relationship between moderate to vigorous physical activity (MVPA) and thiobarbituric acid reactive substances (TBARS) in the group of men ($r = -0.496$; $p < 0.05$).

On the other hand, in the group of men MVPA and CPM were negatively correlated with TBARS test, indicating a decrease in damage of cell membranes related to more intense physical activity. Also Takahashi et al. (2013a,b) observed that MDA concentrations, measured with TBARS, were negatively correlated with the amount of physical activity, although they observed in women and not in men (Takahashi et al., 2013a).

Our results support the hypothesis that physical activity has beneficial effects on the balance between ROS production, the ability to neutralize and the damage in cellular lipid membranes that they may produce in the elderly.

One of the limitations of this study is the small sample size. Although other studies which reported the correlation between physical activity and markers of oxidative stress had bigger sample sizes, it is important to notice that they measured self-reported physical activity which has recently been considered as unacceptable (Dhurandhar et al., 2014). The strength of this study is that physical activity was measured objectively using accelerometers and that its sample size is one of the biggest among studies that correlated objectively measured physical activity and markers of oxidative stress.

5. Conclusions

The findings of our study suggest that MVPA may have a protective effect against oxidative stress by increasing activities of antioxidant enzymes and decreasing membrane lipid peroxidation more than lower intensities of physical activity among older adults.

Conflict of interest

All authors declare that they have no conflict of interest.

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