



ORIGINAL

Physical activity below the minimum international recommendations improves oxidative stress, ADMA levels, resting heart rate and small artery endothelial function



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KEYWORDS

Physical activity;
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Abstract

Background: A moderate level of physical activity (PA), such as a daily 30-min walk, reduces cardiovascular risk. There is a lack of evidence about the cardiovascular benefits of PA below this recommendation of minimum PA level.

Objective: We aimed to study the impact of a lower level of PA on cardiovascular health.

Design: Sixty-four overweight/obese men and women were enrolled in a community programme consisting of 4 months of 1 h, low-intensity PA two days per week. Before and after the intervention, PA level (METs/h/wk), endogenous antioxidant status (SOD and GPX concentration and activity and oxidised LDL), ADMA concentrations, endothelial function by small artery reactive hyperaemia index (saRHI), and resting heart rate (RHR) were assessed.

Results: After the intervention, significant increases in saRHI ($P=0.031$), SOD and GPX activities, and a decrease in ADMA plasma concentrations, and RHR ($P<0.001$ for all) were observed. Increases in PA were positively associated with increases in saRHI ($r=0.341$, $P=0.022$), GPx ($r=0.303$, $P=0.047$) and decreases in RHR ($r=-0.302$, $P=0.047$). Multivariate analyses showed

Abbreviations: PA, physical activity; METs/h/wk, hours/week at a given metabolic equivalent level; SOD, superoxide dismutase; GPx, glutathion peroxidase; ADMA, asymmetrical dimethylarginine; SaRHI, small artery reactive hyperemia index; RHR, resting heart rate; NO, nitric oxide; eNOS, endothelial nitric oxide synthase; ROS, reactive oxygen species; CVR, cardiovascular risk; PAT, peripheral artery tonometry; Alx, augmentation index; Alx@75, augmentation index adjusted to 75 beats per minute; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue; FABP4, fatty acid binding protein 4.

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PALABRAS CLAVE

Actividad física;
Estrés oxidativo;
Función endotelial de
pequeñas arterias;
ADMA

that independent predictors of saRHI improvement were an increase in PA (2.65, 95%CI: 1.21–4.01), decrease in RHR (1.91, 95%CI: 1.01–4.98), and an increase in GPx (2.61, 95%CI: 1.16–5.01).

Conclusion: In obese and overweight men and women, an increase in PA, even below the minimal international recommendations, improves antioxidant capacity, RHR and peripheral small artery reactivity.

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La actividad física por debajo de las recomendaciones internacionales mínimas mejora el estrés oxidativo, los niveles de ADMA, la frecuencia cardíaca en reposo y la función endotelial de las arterias pequeñas

Resumen

Introducción: Los niveles moderados de actividad física (AF), 30 min al día de caminar, reducen el riesgo cardiovascular. No existe evidencia si los niveles bajos de actividad física, por debajo de las recomendaciones internacionales, afectan la salud cardiovascular.

Objetivo: Estudiar el efecto de los niveles bajos de actividad física sobre la salud cardiovascular

Diseño: Se seleccionaron 64 hombres y mujeres con sobrepeso u obesidad para completar un programa comunitario de actividad física consistente en 1 h, 2 días a la semana, durante 4 meses, de AF de intensidad baja. Antes y después del programa se evaluó la AF (MET/h/semana), el estado antioxidante endógeno (SOD y GPX concentración y actividad), las concentraciones de ADMA, la función endotelial de pequeña arteria mediante el índice de hiperemia reactiva (saRHI) y la frecuencia cardíaca (FC) en reposo.

Resultados: Después de la intervención se observó un aumento significativo en el saRHI ($p=0,031$), en la actividad de la SOD y la GPX, y una disminución de las concentraciones plasmáticas de ADMA y de la FC ($p<0,001$ para todos). El aumento en la AF se asoció directamente con el aumento del saRHI ($r=0,341$, $p=0,022$), GPx ($r=0,303$, $p=0,047$) y disminución en FC ($r=-0,297$, $p=0,047$). Los predictores independientes de la mejora del saRHI fueron un aumento en la AF (2,65; IC 95%: 1,21-4,01), la disminución de la FC (1,91; IC 95%: 1,01-4,98) y el aumento de la GPx (2,61; IC 95%: 1,16-5,01).

Conclusiones: Un aumento de la AF, incluso por debajo de las recomendaciones internacionales de AF, mejoró la capacidad antioxidante, la FC y la función endotelial de las pequeñas arterias en hombres y mujeres con sobrepeso u obesidad.

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Introduction

Regular physical activity (PA) is associated with a reduced risk of cardiovascular events and is therefore a central component in primary and secondary prevention of cardiovascular disease.^{1,2} Several major scientific bodies currently recommend and encourage a minimum of 2.5–5 h/week of physical activity or aerobic exercise training of at least moderate intensity or 1–2.5 h/week of vigorous intensity exercise.^{3,4}

Among the benefits on classical cardiovascular risk factors, increasing physical activity modulates oxidative stress and nitric oxide (NO) bioavailability, both of which contribute to improved endothelial function and reduced cardiovascular risk.^{3–6} It is known that sedentary men and women have impaired endothelium-dependent dilatation. Endothelial function is carefully controlled, mainly by the bioavailability of NO and sympathetic and parasympathetic drive reflexes.⁷ NO bioavailability is in part the result of

the balance between the production and the neutralisation of reactive oxygen species (ROS). In this context, increased concentrations of asymmetrical dimethylarginine (ADMA), an endogenous L-arginine analogue, may uncouple endothelial NO synthase (eNOS), which impairs NO production but also increases superoxide production in the vascular endothelium.⁸ Human and animal data show a clear association between ROS production and the activation of the sympathetic drive, which may cause impairment of NO release and function.^{9,10} Although physical activity has a clear positive impact on cardiovascular risk, some types and levels of PA have been associated with increased oxidation in the short term and also the impact on endothelial function is controversial.^{11,12} Moreover, reported low adherence to physical activity interventions in large community-based studies reflects the difficulty of implementing a physical activity plan.^{13,14} This low adherence also contributes to the differences in the above mentioned studies. Engaging chronically sedentary groups who have physical boundaries such

as ageing and obesity into a PA programme is rather difficult. A low intensity PA programme could increase adherence. However, the real impact of this level of PA on cardiovascular performance and intermediate factors for cardiovascular risk (CVR), such as oxidative stress and endothelial function, have not been assessed. In this work, we aim to determine whether implementing a low-intensity physical activity programme in a group of sedentary overweight/obese men and women has any impact on oxidative stress and small artery vascular function.

Materials and methods

Design, population, clinical assessment and intervention

This is a nested study from a primary care community programme ‘‘Step by Step’’ involving all of the primary care centres in the city of Reus (Spain). Participants in this programme are men and women at increased cardiovascular risk or with established cardiovascular disease. The aim of this community programme is to boost a healthy lifestyle in people at increased cardiovascular risk. It consists of 1 h low-intensity walking sessions twice a week. These sessions are directed by health care and physical education professionals. Before every session, participants signed an attendance list.

The inclusion criteria for the present study were men and women aged between 30 and 70 years with increased cardiovascular risk who were overweight, defined as body mass index between 25 and 30 kg/m², or obese, defined as a body mass index higher than 30 kg/m² or waist circumference higher than 102 cm in men and 88 cm in women as specified by the ATP III clinical criteria for abdominal obesity. Exclusion criteria were as follows: unwillingness to cooperate in the study; severe disease that may interfere with the ability to comply with study protocol; shorten life expectancy; and patients diagnosed with neoplasia or cardiovascular disease. Out of 211 eligible volunteers of the Step by Step programme, sixty-four fulfilled the inclusion criteria and were included in this study.

A complete physical examination including lifestyle components (diet, physical activity and smoking), anthropometry assessment, and biochemical, abdominal fat and vascular studies (small artery reactive hyperaemia index, saRHI) were performed before and after the 4-month follow-up period. Leisure-time physical activity was quantified as Metabolic Equivalents (METs/week) using the Minnesota questionnaire adapted for the Spanish population.¹⁵ A validated semi-quantitative food frequency questionnaire was used to evaluate diet.¹⁶ Tobacco status was assessed using standardised questionnaires administered by health care providers.¹⁷ The patients’ usual pharmacological treatment was maintained. The sample size considering an 80% power to detect a difference between means of 0.04 in saRHI, 0.02 in ADMA and 0.01 in SOD and Gpx, with a level of significance (alpha) of 0.05 (two-tailed) was calculated regarding these conditions. The sample size required was 45 participants. This study was conducted according to the guidelines of the Declaration of Helsinki, and all procedures involving human patients were approved by the Ethical and Clinical

Investigation Committee of the hospital. Written informed consent was obtained from all patients.

Resting heart rate and small artery function measurement

Resting heart rate (RHR) and saRHI were measured using peripheral artery tonometry (PAT) technology (EndoPAT-2000 device, Itamar Medical Ltd., Israel). Both measurements were performed in a quiet room with a controlled temperature (22–24°C) after patients had fasted for 12 h and refrained from smoking or strenuous exercise for 24 h. The patients lay in a relaxed, quiet and evenly illuminated environment while the device recorded changes in pulse waves in the digital arteries. The PAT technique has been described elsewhere.¹⁸ There is a 5 min baseline measurement, after which the cuff is inflated enough above baseline systolic pressure to produce ischaemia. After 5 min of occlusion, the cuff is rapidly deflated and the PAT tracing is recorded for an additional 5 min. Blood flow measurements from two fingertips on different hands—one a test, the other a control—were compared after a stabilisation period. A second pair of measurements for comparison was taken before and after five minutes of brachial ischaemia in the test arm. The results were processed with specific software to calculate the post-ischaemia reflex vasodilatation observed when measurements from the test arm (before and after ischaemia) were compared to those from the control arm. The value generated was termed saRHI. Arterial stiffness, measured as augmentation index (Alx), was also determined during the same exploration and processed by specific software by analysing the differences in pulse wave amplitude before and after ischaemia in comparison to the control arm. We then calculated the Alx adjusted to 75 beats per minute variable (Alx@75).

Subcutaneous and visceral fat determination

A MyLab 50 X-Vision ultrasonograph (Esaote, Italy) equipped with a linear array ultrasound probe (7.5–12 MHz) was used to assess body fat according to the ultrasound image review consensus.¹⁹ Briefly, the thicknesses of subcutaneous and preperitoneal fat were measured by placing the probe perpendicular to the skin on the epigastrium. The thickness of the subcutaneous fat is defined as the distance between the anterior surface of linea alba and the fat-skin barrier. The preperitoneal fat extends from the anterior surface of the liver (left lobe) to the posterior surface of linea alba.

Biochemical determinations

Standard biochemical parameters were determined via the usual methods. Glucose, cholesterol, triglyceride, apolipoprotein B100, apolipoprotein A1, direct LDL-cholesterol, HDL-cholesterol, and high-sensitivity C-reactive protein (hs-CRP) were measured using enzymatic and immunoturbidimetric assays (Spinreact, SA, Spain and Wako Chemicals GmbH, Germany) adapted to the Cobas Mira Plus autoanalyser (Roche Diagnostics, Spain). SOD and GPX plasma and erythrocyte lysate levels were determined by

commercially available assays according to the manufacturer's instructions (Cayman Chemical Company, USA). ADMA and LDLox plasma levels were determined by commercially available immunoassays according to manufacturer's instructions (ADMA-ELISA, Immunodiagnostik AG, Germany; oxLDL-ELISA, Mercodia, Sweden). FABP4 levels were determined with commercial ELISA kits (Bio Vendor Laboratory Medicine Inc., Brno, Czech Republic).

Statistical analyses

Data are presented as the mean $\pm$ SD or as the median and interquartile range for continuous variables and as frequencies for categorical variables. Normality of the distribution of variables was assessed with the Kolmogorov-Smirnov test. Differences between physical activity, anthropometric, biochemical or vascular data were analysed using the ANOVA or Kruskal-Wallis test for continuous variables or with the chi-squared test for categorical variables. Univariate association was tested by Spearman correlation analysis. A multiple stepwise logistic regression test was performed to assess the determinants of saRHI in our study group patients. The dependent variable of interest was the saRHI. Independent variables were selected based on univariate analysis and from the variables known to be associated with the dependent variable according our previous studies. In this test, independent variables were age, smoking, increase in METs/h/wk, decrease in waist circumference, GPX, SOD, RHR and ADMA changes. *P*-values were calculated as two-sided; a *P*-value of less than 0.05 was considered statistically significant. SPSS version 19.0 (SPSS Inc., Chicago, IL) was used for all statistical analysis.

Results

Fifty-four participants completed the study protocol (Fig. 1). Mean attendance at physical activity sessions was 74%. After the intervention, an increase in the number of METs/h/wk (7.7 ± 1.9 vs. 17.2 ± 1.5 , $P < 0.001$) was observed. Anthropometric, diet, body fat distribution and general biochemical characteristics did not change during the intervention (Table 1).

Variations in antioxidant enzymes and vascular determinants between baseline and end of the intervention are shown in Table 2. We observed a significant increase in intra-erythrocyte and plasma SOD concentrations (SODe; 2096 ± 890 vs. 3326 ± 1013 U/grHb, $P < 0.001$ and SODa; 3.8 ± 1.4 vs. 4.6 ± 1.8 Ummol, $P = 0.011$) and both, intra-erythrocyte GPxe (9579 ± 3384 vs. $12,488 \pm 2405$ μ mol/min/grHb, $P < 0.001$). The saRHI increased (1.91 ± 0.47 vs. 2.31 ± 0.51 , $P = 0.031$) correspondingly to a significant decrease of ADMA levels (0.47 ± 0.11 vs. 0.40 ± 0.09 mol/min/grHb, $P < 0.001$) and GPX activity (40 ± 12 vs. 35 ± 16 μ mol/min/m, $P = 0.044$). LDLox did not change significantly (85 ± 31 vs. 77 ± 30 U/L, $P = 0.232$). RHR decreased by $8.6 \pm 19.7\%$ after the intervention ($P < 0.001$).

Univariate tests showed that the increase in PA was positively associated with the increase in saRHI ($r = 0.341$, $P = 0.022$) (Fig. 2a) and GPxe ($r = 0.303$, $P = 0.047$). Moreover, PA inversely correlated with changes in glucose ($r = -0.302$,

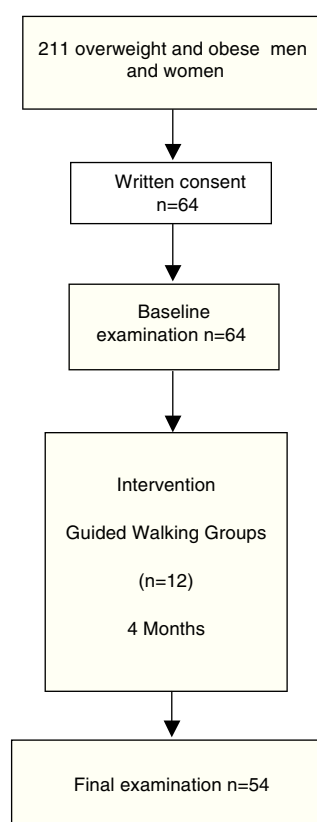


Figure 1 Participants flow diagram.

$P = 0.047$), body weight ($r = -0.441$, $P = 0.004$) and resting heart rate ($r = -0.319$, $P = 0.041$).

Changes in saRHI inversely correlated to changes in RHR ($r = -0.360$, $P = 0.021$) (Fig. 2b). Participants with greater increases in saRHI had greater increases in SODE levels compared to those with smaller increases in saRHI (2134 ± 411 vs. 326 ± 286 U/grHb, $P = 0.04$).

A multiple stepwise logistic regression test was performed to assess the main determinants of endothelial function improvement, using saRHI increase as the dependent variable. The independent variables included were age, smoking, increase in METs/h/wk, decrease in waist circumference, GPxe increase, SODE increase, changes in RHR and ADMA decrease. After adjusting for interactions, the best predictor model (85.4% of correctly prognosticated values and R^2 Nagelkerke = 0.442) included smoking, increase in METs/h/wk, changes in RHR and increase in GPxe. When this test was forced, the increase in METs/h/wk (2.65 95%CI: 1.21–4.01), changes in RHR (1.91 95%CI: 1.01–4.98) and increase in GPxe (2.61 95%CI: 1.16–5.01) remained independent predictors of saRHI improvement (Fig. 3).

Discussion

Our work shows that low-intensity level PA applied to sedentary people improves several parameters associated with cardiovascular health and that there is high adherence to such a physical activity programme. International recommendations by different scientific societies establish a minimum of 2.5–5 h a week of moderate intensity walking

Table 1 Differences in clinical, anthropometrical and biochemical data between baseline and post-intervention.

	Baseline (n = 64)	After intervention (n = 54)	P-value
Age, years	62 ± 8		
Smokers (%)	12.7		
Obesity (%)	52.7		
DM2 (%)	9.1		
Hypertensive (%)	36.4		
METs/h/wk	7.7 ± 1.9	17.2 ± 1.5	<0.001
Mediterranean diet score (u)	30 ± 3	31 ± 4	0.633
Body weight (kg)	74.1 ± 8.4	74.2 ± 8.3	0.958
BMI (kg/m ²)	30.11 ± 4.58	30.06 ± 4.82	0.768
Waist circumference (cm)	99 ± 13	98 ± 14	0.328
SAT (mm) ^a	18 ± 2.5	14 ± 2.8	0.205
VAT (mm) ^a	15 ± 6	14 ± 6	0.393
Systolic blood pressure (mmHg) ^a	132 ± 15	136 ± 17	0.133
Total cholesterol (mmol L ⁻¹)	5.36 ± 1.17	5.68 ± 1.03	0.793
LDLc (mmol L ⁻¹)	3.45 ± 1.07	3.48 ± 0.87	0.797
HDLc (mmol L ⁻¹) ^a	1.25 ± 0.41	1.31 ± 0.44	0.571
Apolipoprotein B100 (mmol L ⁻¹)	1.21 ± 0.29	1.21 ± 0.27	0.984
Apolipoprotein A1 (mmol L ⁻¹)	1.54 ± 0.33	1.61 ± 0.26	0.176
Triglyceride (mmol L ⁻¹) ^a	1.35 ± 0.74	1.28 ± 0.73	0.941
Glucose (mmol L ⁻¹)	5.57 ± 1.21	5.68 ± 1.44	0.140
FABP4 (ng/ml)	33.1 ± 13.5	31.1 ± 13.1	0.521
HsCRP (mg L ⁻¹) ^a	3.98 ± 5.11	3.71 ± 4.53	0.560

SAT: Subcutaneous adipose tissue; VAT: Visceral adipose tissue. FABP4: Fatty acid binding protein 4; HsCRP: High sensitive C-reactive protein.

Derived with an ANOVA or Wilcoxon test for continuous variables or chi-squared test for categorical variables.

^a Values are given as the mean ± SD, percentage of patients or median ± interquartile range.

to reduce cardiovascular risk.^{3,4} However, even this small amount of activity cannot be met by many elderly, handicapped or metabolically limited people, such as those with obesity. Therefore, an evaluation of the impact of exercise levels below these recommendations is warranted. A low PA intervention results in an augmented antioxidant system measured by the activity of the enzymes SOD and GPX. The increases in these enzymes suggest a better antioxidant status, although we did not observe any effect on plasma lipid oxidation as measured with oxidised LDL levels. The impact

of low-level PA on peripheral small artery endothelial function was also significant. Moreover, our data also show that in parallel with saRHI increase, there was a significant decrease in ADMA levels.

Current evidence suggests that PA improves several classical cardiovascular risk factors as well as measures of new biomarkers of cardiovascular risk, including oxidative stress,^{20,21} endothelial wall dysfunction^{5,6,11,21} and high resting heart rate.²² It is well known that weight loss, especially visceral fat loss,²³ but also subcutaneous fat loss,²⁴ is one

Table 2 Oxidative and vascular differences between baseline and after the intervention.

	Baseline (n=64)	After intervention (n = 51)	P-value
SODc (U/grHb) ^a	2096 ± 890	3326 ± 1013	<0.001
SODa (U/mmol)	3.8 ± 1.4	4.6 ± 1.8	0.011
GPXc (umol/min/grHb) ^a	9579 ± 3384	12,485 ± 2405	<0.001
GpXa (umol/min/ml)	40 ± 12	35 ± 16	0.044
ADMA (μmol/L) ^a	0.47 ± 0.11	0.40 ± 0.09	<0.001
LDLox (U/L) ^a	85 ± 31	77 ± 30	0.232
SaRHI (u) ^a	1.91 ± 0.47	2.31 ± 0.51	0.031
Resting heart rate, bpm	71 ± 11	64 ± 9	<0.001
Alx@75 (u) ^a	27.5 ± 14.2	30.1 ± 17.7	0.191

SODc: Superoxide dismutase erythrocyte lysate levels; SODa: Superoxide dismutase plasma; GPXc: Glutathion peroxidase erythrocyte lysate levels; GPXa: Glutathion peroxidase plasma; ADMA: asymmetrical dimethylarginine; LDLox: Oxidised LDL cholesterol; saRHI: small artery reactive hyperemia index; Alx@75: Augmentation index adjusted to 75 bpm.

Baseline compared with after intervention with a paired *t*-test or Wilcoxon test for continuous variables.

^a Values are given as the mean ± SD or median ± interquartile range.

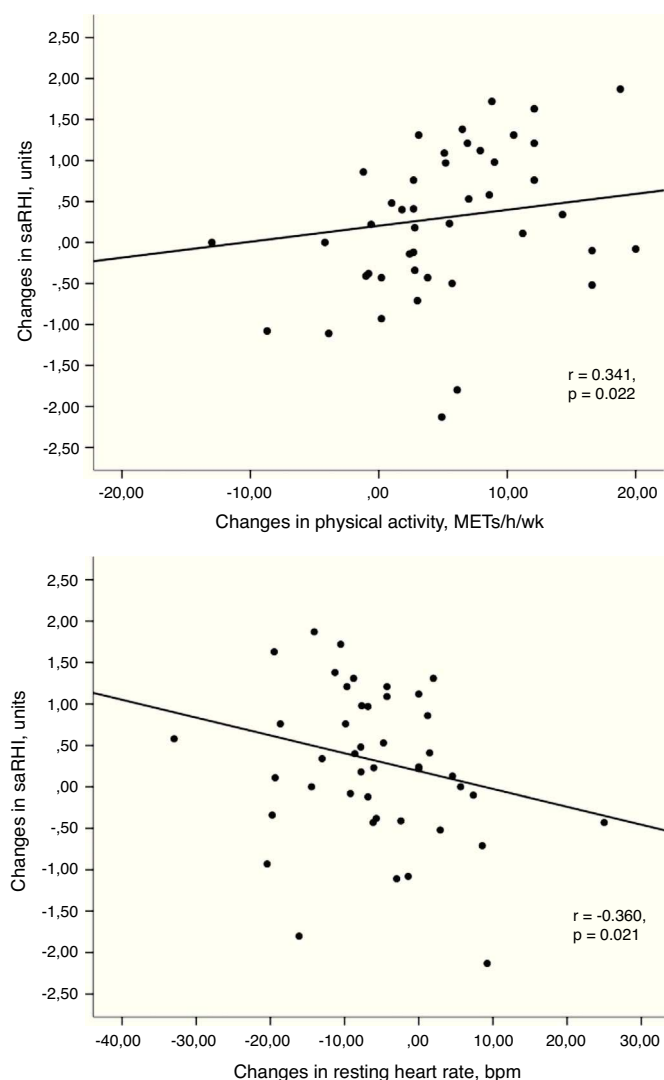


Figure 2 (a) Association between changes in small artery reactive hyperemia and changes in physical activity. Univariate associations derived from Spearman's correlation test. (b) Association between changes in small artery reactive hyperemia and changes in resting heart rate. Univariate associations derived from Spearman's correlation test.

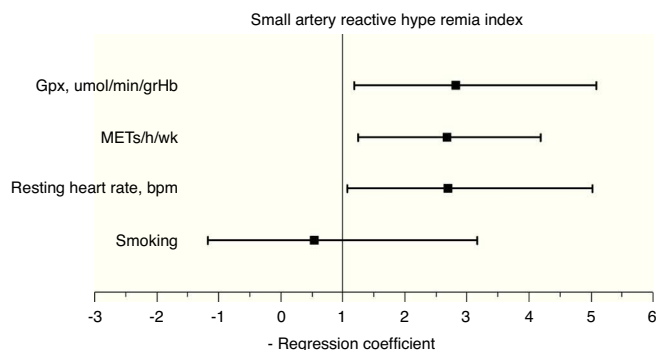


Figure 3 Determinants of small artery reactive hyperemia. Dependent variable: saRHI: small artery reactive hyperemia index changes between baseline and after the intervention. Independent variables: smoking, increase in METs/h/wk, changes in RHR, increase in GPxe.

of the main determinants of these changes. By assessing body fat patterns with standardised echography methods, we observed that our results were independent of changes in body weight loss or body fat distribution. Moreover, our group has recently published that a moderate increase in physical activity decreases a marker of adipose tissue distribution like fatty acid binding protein 4 (FABP4) levels; specifically, an increase of 30 METs/h/wk decreases FABP4 by 10.3 units.²⁵ In the present study, the low increase in physical activity has not been associated with FABP4 changes.

We found that ADMA levels decreased significantly with low intensity PA. Exercise training has been documented to decrease ADMA levels,²⁶ but no data are available for low intensity PA. ADMA acts as strong endogenous eNOS inhibitor by blocking NO synthesis from the amino acid L-arginine, which contributes to the initiation and progression of atherosclerosis.⁸ Therefore, the suppression of ADMA and the increase of saRHI might also reflect improved endothelial function in overweight and obese men and women undergoing low intensity PA. Even though we cannot obtain mechanistic data from this study, our results are very consistent in showing both an improvement in oxidative status and small artery endothelial function markers. These results were confirmed in multivariate analyses showing that both PA and antioxidant enzymes play a role in the observed endothelial function improvement.

Another relevant finding is the significant decrease in RHR observed after the intervention. High resting heart rate has been identified as a potential accelerator of atherosclerosis via its negative effects on the endothelium due to shear and mechanical stress. In fact, modifications of the haemodynamic environment by accelerated heart rate likely contributes to enhanced atherosclerosis in certain parts of the vascular tree.²⁷ It is known that physical exercise practice improves heart function by reducing RHR; however, it is important to note that small amounts of PA already have a positive impact. Interestingly, changes in RHR were among the determinants of saRHI improvement, which supports the idea that heart functionality is linked to overall endothelial function. In accordance with this observation, clinical trials with ivabradine, a selective inhibitor of the $I_{(f)}$ channels, showed a relationship between decreasing RHR and improved endothelial function.^{27,28} This association is not fully understood, but evidence derived from ivabradine studies produced two possible explanations of this effect. The first is that by slowing heart rate, shear stress is more constant and thus flow is more constant, leading to a reduced burden on the arterial wall function.²⁹ The second is associated to the mechanical stress from each heart-beat. At a high RHR, the wall becomes stiffer; this is associated with accelerated endothelial cell turnover, premature senescence of the endothelium and high shear stresses.³⁰ Our results support these observations and extend them to clinical practice, suggesting a benefit of low intensity PA in obese or overweight postmenopausal women.

Study limitations

Our study had some limitations. Because the study was conducted in a specific group of overweight or obese men and women, results cannot be generalised to other

specific populations. Although not described, either the pharmacological agents our patients were taking, or interactions between pharmacological agents and physical activity changes, could have influenced changes in oxidative stress or endothelial function. Physical activity is measured objectively by self-reports that may lead to misclassification and bias towards weaker physical-activity or health-benefit associations. In view of the strength of our main findings, the quantitative importance of these limitations appears small.

Conclusions

The results of the present study suggest that lower-intensity PA improves markers of cardiovascular health such as oxidative stress, RHR and peripheral small artery wall function in a group of obese and overweight men and women. These changes are independent of weight loss or fat modifications suggesting a direct impact of low amounts of PA on cardiovascular health improvement. New long-term clinical trials with other specific populations are needed to establish clinical recommendations in specific handicapped study populations at increased cardiovascular risk.

Ethical disclosures

Protection of human and animal subjects. The authors declare that no experiments were performed on humans or animals for this investigation.

Confidentiality of Data. The authors declare that no patient data appears in this article.

Right to privacy and informed consent. The authors have obtained the informed consent of the patients and /or subjects mentioned in the article. The author for correspondence is in possession of this document.

Conflict of interest

The authors declared no conflict of interest.

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