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PHYSICAL ACTIVITY AND HEALTH

The Effect of Physical Activity on Arterial Stiffness, Inflammation and Lipoproteins among 30–65-Year-Old Men

RESEARCH

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HARRI J. SAARINEN
HANNA POHJANTÄHTI-MAAROOS
RIITTA ANTIKAINEN
JORMA T. LAHTELA
ARI PALOMÄKI

*Author affiliations can be found in the back matter of this article

ABSTRACT

Introduction: Obesity and an inactive lifestyle increase risk for metabolic syndrome and cardiovascular disease. We did a cross-sectional study on 120 men with and 80 men without metabolic syndrome to investigate the connection between patient-reported physical activity and cardiovascular risk factors.

Methods: The subjects' daily amount of physical exercise was assessed with a structured questionnaire regarding normal weekly amount, type and mode of physical activity. The subjects were stratified into different groups regarding their physical activity and metabolic syndrome status. We compared lipid levels, resting heart rate, hs-CRP, HbA1c and arterial elasticity of the subjects in different groups.

Results: Subjects with metabolic syndrome and high amounts of daily physical activity had higher resting heart rate, hs-CRP, triglycerides and lower HDL cholesterol than their counterparts without metabolic syndrome who reported lower levels of daily physical activity.

Conclusions: An increase in daily physical activity was associated with lower HbA1c among men with metabolic syndrome up to the level of subjects without metabolic syndrome. Increasing physical activity solely is an insufficient strategy for improving all the unfavorable cardiovascular risk factors related to metabolic syndrome.

CORRESPONDING AUTHOR:
Harri J. Saarinen
Tampere Heart Hospital, FI
harri.saarinen@fimnet.fi

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metabolic syndrome; physical activity; arterial stiffness; inflammation; diabetes; cholesterol

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INTRODUCTION

Metabolic syndrome (MetS) is a combination of cardiovascular disease (CVD) risk factors such as hypertension, dyslipidemia, visceral obesity and abnormal glucose tolerance or diabetes (NCEP, 2001). CVD is the leading cause of death globally (World Health Organization, 2020) and MetS is associated with a twofold increase in CVD outcomes and 1.5-fold increase in all-cause mortality (Mottillo et al., 2010).

Smoking cessation, dietary changes and physical activity (PA) form the basis of lifestyle intervention for CVD risk factors and lifestyle interventions should be applied prior to or at least together with CVD preventive medical therapies to improve the outcome.

Aerobic exercise training reduces triglycerides (TG) and increases high-density lipoprotein cholesterol (HDL-C) in primary prevention setting among subjects who have previously been sedentary and have CVD risk factors (Lin et al., 2015). Regular PA helps to lose weight particularly when combined with reduced calorie intake and prevents weight regain after initial weight loss (Piercy et al., 2018). Even a single episode of moderate-to-vigorous PA can reduce blood pressure (BP) and improve insulin sensitivity on the day of the performed activity. These effects improve with regular performance of PA (Piercy et al., 2018).

Prevention of CVD and slowing down the progression of subclinical atherosclerosis has focused heavily on blood cholesterol levels, glycemic status and BP, but there are also other CVD risk factors such as high resting heart rate (RHR), arterial stiffness and chronic inflammation (Zhang, Shen & Qi, 2016; Arnett et al., 2019). Arterial stiffness may also serve as a useful biomarker for predicting CVD risk in patients close to decisional thresholds, although its systematic use among general population is not recommended (Piepoli et al., 2016). PA has been shown to have a positive effect on resting heart rate (Reimers, Knapp and Reimers, 2018) and arterial stiffness (Vaitkevicius et al., 1993). The physical activity guidelines for Americans recommend that for substantial health benefits adults should aim for 150–300 minutes a week of moderate-intensity PA or 75 to 150 minutes of vigorous-intensity aerobic PA (Piercy et al., 2018). However, instructing the patients regarding the intensity, duration and frequency of PA can be difficult since the patients' subjective estimation especially of the intensity of their PA can vary substantially and depends on their physical abilities and sports history.

The objective of this study was to assess the effect of PA on arterial stiffness, cholesterol, inflammation, glucose metabolism and RHR among 30–65-year-old men with and without MetS and different amount of PA.

METHODS

SUBJECTS

The data for this cross-sectional study is derived from the Hämeenlinna Metabolic Syndrome Research Program, which investigates atherosclerotic risk factors in men with MetS (Pohjantahti-Maaroos et al., 2012). The study subjects were 120 men with MetS diagnosed in primary health care according to the National Cholesterol Education Program (NCEP) Adult Treatment Panel III criteria (NCEP, 2001) (MetS group) and a control group of 80 physically active non-MetS men (Non-MetS group) with age from 30 to 65 years. The control group were men from Hämeenlinna region who were enrolled in the study from local recreational activity clubs and through local newspaper advert.

Each subject's weight, height, waist circumference and BP were measured in physical examination. Smoking habits and medication were gathered with a standardized interview. The subjects filled out a questionnaire regarding their normal weekly amount, type and mode of PA. Only leisure time PA was taken into account and work-related PA was discarded from this study. The estimated energy expenditure of weekly PA was calculated with a method described by Aittasalo, Miilunpalo & Suni (2004). In short, we multiplied the mean duration of exercise in hours with exercise times per week and the subject's weight in kilograms and the metabolic equivalent of task (MET) intensity level of the activity. This was finally divided by seven to get the mean daily amount of physical exercise in kilocalories. The correct MET intensity level for specific activities was selected from the list provided by Ainsworth et al. (2011) utilizing the patient's self-rated intensity level of exercise.

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The subjects with and without MetS were handled in separate groups and then both groups were further stratified into three more groups based on their daily amount of physical exercise: subjects with a daily amount of less than 100 kcal PA were termed sedentary, subjects with 100–300 kcal of daily PA were termed Moderate physical activity (MPA) group and subjects with more than 300 kcal of daily PA were termed High physical activity (HPA) group. For a middle-aged man who weighs 85 kg, 100 kcal of leisure-time PA would equal 72 minutes of jogging a week. 300 kcal of leisure-time PA would equal to one 1.5 h game of soccer in addition to 83 minutes of jogging a week.

From a total number of 200 subjects, we excluded 12 from the non-MetS group for insufficient filling of physical activity questionnaire. Sixty-six subjects with MetS reported none or less than 100 kcal of daily PA and were classified in the sedentary group. Only one subject in the non-MetS group was classified as sedentary and was not included in the analysis. A total of 187 subjects were included in the final analysis and 120 of them had MetS (Figure 1). The baseline characteristics of the subjects are presented in Table 1.

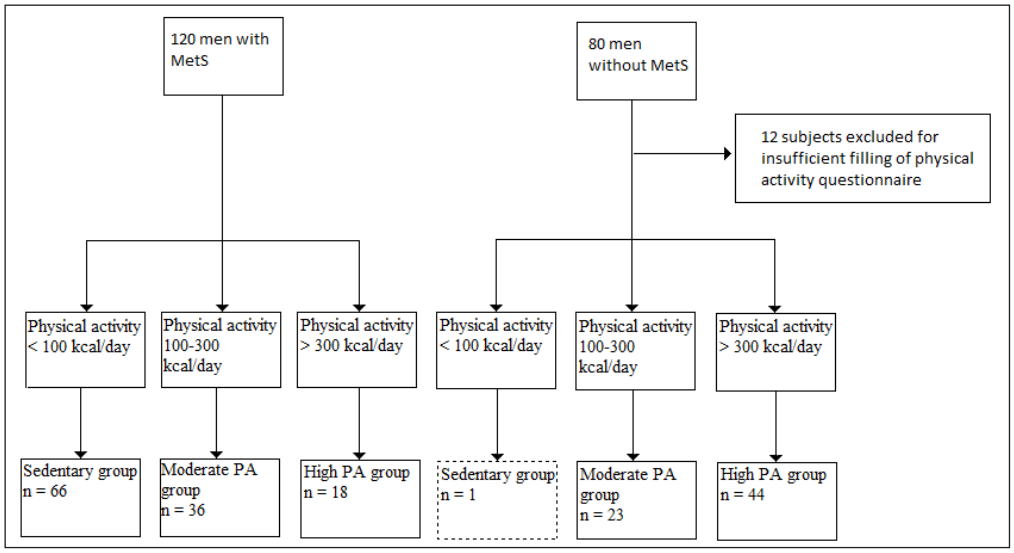


Figure 1 The study inclusion/exclusion flowchart. MetS = Metabolic syndrome PA = physical activity.

Table 1 Baseline characteristics.

The values are presented as number (%) and mean ± standard deviation. MetS: Metabolic Syndrome BMI: Body mass index ACE: Angiotensin-converting enzyme ARB: Angiotensin II receptor blockers SBP: Systolic blood pressure DBP: Diastolic blood pressure MPA: Moderate physical activity group HPA: High physical activity group.

ACTIVITY GROUP	GLYCEMIC STATUS				
	METS n = 120			NON-METS n = 67	
	SEDENTARY n = 66	MPA n = 36	HPA n = 18	MPA n = 23	HPA n = 44
Age, years	49.5 ± 8.1	52.0 ± 8.0	51.8 ± 8.5	52.9 ± 5.6	52.3 ± 8.9
Physical activity, kcal/day	24.1 ± 32.1	175.5 ± 42.4	529.6 ± 178.1	209.0 ± 65.0	604.6 ± 262.8
Waist circumference, cm	113.0 ± 11.0	113.1 ± 12.6	113.9 ± 17.3	90.0 ± 7.4	89.4 ± 7.1
BMI, kg/m²	32.0 ± 4.6	31.9 ± 5.4	31.8 ± 5.6	24.1 ± 2.2	24.5 ± 2.2
Medication					
• aspirin, n (%)	15(28%)	10 (28%)	4 (22%)	3 (13%)	2 (5%)
• beta-blockers, n (%)	24 (36%)	14 (39%)	4 (22%)	1 (4%)	0 (0%)
• ACE-inhibitors, n (%)	11 (17%)	6 (17%)	3 (17%)	1 (4%)	2 (5%)
• ARBs, n (%)	19 (29%)	7 (19%)	0 (0%)	0 (0%)	1 (2%)
• statins, n (%)	21 (32%)	9 (25%)	5 (28%)	4 (17%)	4 (9%)
• glucose-lowering drugs, n (%)	13 (20%)	3 (8%)	2 (11%)	0 (0%)	0 (0%)
Smoking					
• current, n (%)	19 (29%)	4 (11%)	2 (11%)	1 (4%)	0 (0%)
• former, n (%)	29 (44%)	18 (50%)	10 (56%)	6 (26%)	19 (43%)
• never, n (%)	18 (27%)	14 (39%)	6 (33%)	16 (70%)	25 (57%)
• Pack-years in smokers	17.9 ± 15.9	17.1 ± 11.9	13.3 ± 11.4	4.1 ± 3.0	9.0 ± 8.6
• SBP, mmHg	140.1 ± 12.6	135.1 ± 12.6	143.8 ± 18.6	129.6 ± 15.7	129.4 ± 10.8
• DBP, mmHg	82.8 ± 7.8	79.7 ± 6.0	85.5 ± 11.6	75.6 ± 8.7	75.5 ± 5.7

The study followed the ethical principles of the Declaration of Helsinki and each study subject gave a written informed consent. The study protocol was approved by the Research Ethics Board of Kanta-Häme Hospital District.

BLOOD SAMPLING

Blood samples were collected after a 12-hour overnight fast into 10 mL EDTA tubes, 5 mL lithium-heparin gel tubes and 2 mL natrium-fluoride tubes with a minimum of ten-minute rest. Total cholesterol, LDL-C, HDL-C, TG and high-sensitivity C-reactive protein (hs-CRP) concentrations were analyzed in hospital laboratory by Cobas Integra procedure (Roche). The laboratory practices strict internal quality control with national external quality assurance program (Labquality Oy) performing frequently control samples. The lower threshold for hs-CRP analysis was 0.18 mg/L and values lower than this were simply reported <0.18 mg/L, so the data for hs-CRP was reported as median and interquartile range (IQR). For the rest, the data were reported as mean and standard deviation or number and percentage.

MEASURING OF ARTERIAL ELASTICITY

The arterial elasticity was measured non-invasively with arterial tonometer HDI/PulseWave™ CR-2000 (Hypertension Diagnostics Inc., Eagan, MN, USA). The tonometer records the radial artery pulse wave and uses a modified Windkessel method (Cohn et al., 1995) to report the capacitive elasticity of large arteries (C1) and the reflective elasticity of small arteries (C2) as a mean of five most similar pulse waves that appear during the measuring. The C1 represents the elastic properties of large arteries and the C2 represents the endothelial function of the microvascular circulation (Cohn, 1999). We performed four consecutive measurements of C1 and C2 for each subject to diminish the variability and possible bias caused by single measurement. Higher values indicate higher arterial elasticity. The subjects had at least a 10-minute rest before measurements.

STATISTICAL METHODS

Statistical analysis was done with IBM SPSS Statistics 27. We used Kruskal-Wallis test to analyze the differences between the groups as normality couldn't be reliably assumed because some of the groups were relatively small. A p-value of ≤0.05 was considered to indicate significance. Possible significant correlations between PA and cardiovascular risk factors were tested by Pearson's or Spearman's correlation analysis, as appropriate.

RESULTS

Comparison of the subjects with the same metabolic status but different PA classes showed no difference in hs-CRP, HDL-C, LDL-C, TG, RHR or arterial elasticity (Figures 2A–D, 3A–C). The levels of glycated hemoglobin (HbA1c) were significantly lower in MetS subjects with higher amount of daily PA compared to sedentary group: 43.3 ± 14.4 mmol/mol vs. 46.3 ± 13.1 mmol/mol ($p = 0.046$). There was also a borderline significant difference ($p = 0.050$) in HbA1c between MetS subjects with high and moderate amount of daily PA (43.3 ± 14.4 mmol/mol vs 46.5 ± 11.3 mmol/mol) ($p = 0.050$). This effect was not seen in non-MetS subjects or when MetS subjects from sedentary and MPA groups were compared with each other (Figure 2E). However, there was no significant relationship between daily PA and HbA1c in correlation analysis among all MetS or non-MetS groups combined.

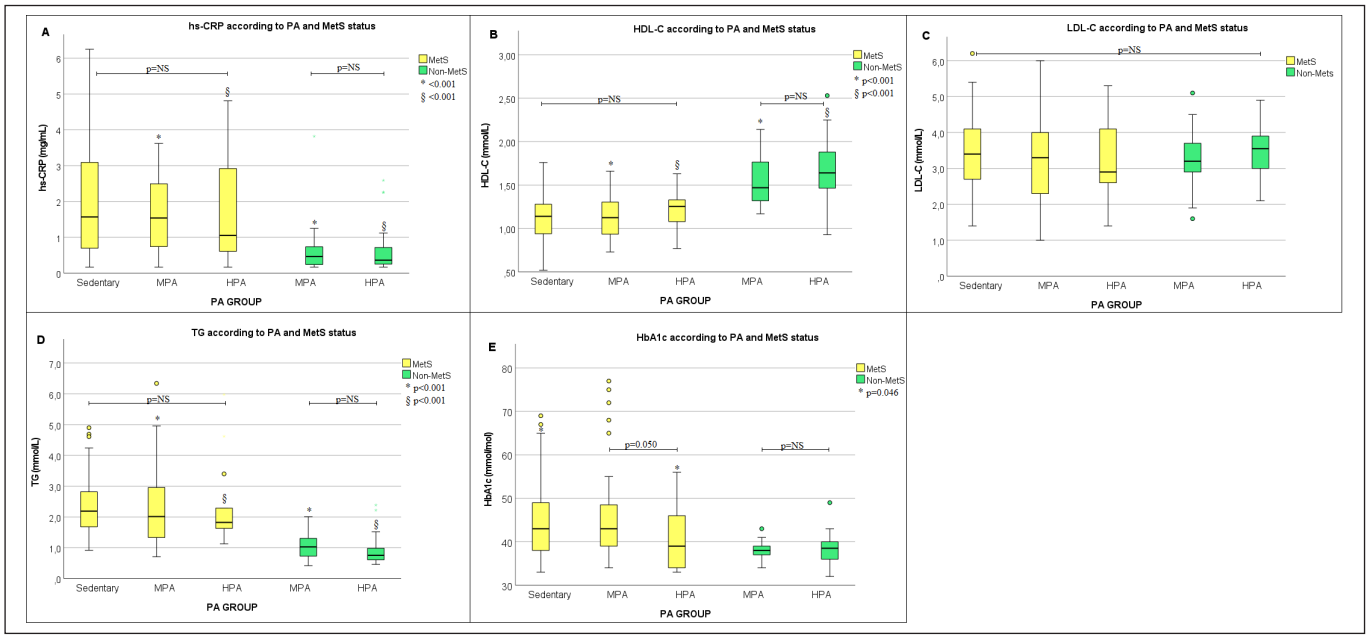
When the PA groups were compared to the group with the same amount of PA but different MetS status the subjects with MetS had higher median hs-CRP in both activity groups compared to non-MetS: 1.5 (1.7) mg/L vs. 0.5 (0.5) mg/L ($p < 0.001$) for MPA groups and 1.1 (2.2) mg/L vs. 0.4 (0.4) mg/L ($p < 0.001$) for HPA groups (Figure 2A). HDL-C concentration was higher among non-MetS subjects than MetS subjects with the same amount of daily PA in both activity groups: 1.6 ± 0.3 mmol/L vs. 1.2 ± 0.3 mmol/L ($p < 0.001$) for MPA groups and 1.7 ± 0.3 mmol/L vs. 1.2 ± 0.2 mmol/L ($p < 0.001$) for HPA groups (Figure 2B). MetS subjects in both activity groups had higher TG than non-MetS subjects: 2.5 ± 1.7 mmol/L vs. 1.1 ± 0.4 mmol/L ($p < 0.001$) for MPA groups and 2.9 ± 3.2 mmol/L vs. 0.9 ± 0.4 mmol/L ($p < 0.001$) for HPA groups (Figure 2D).

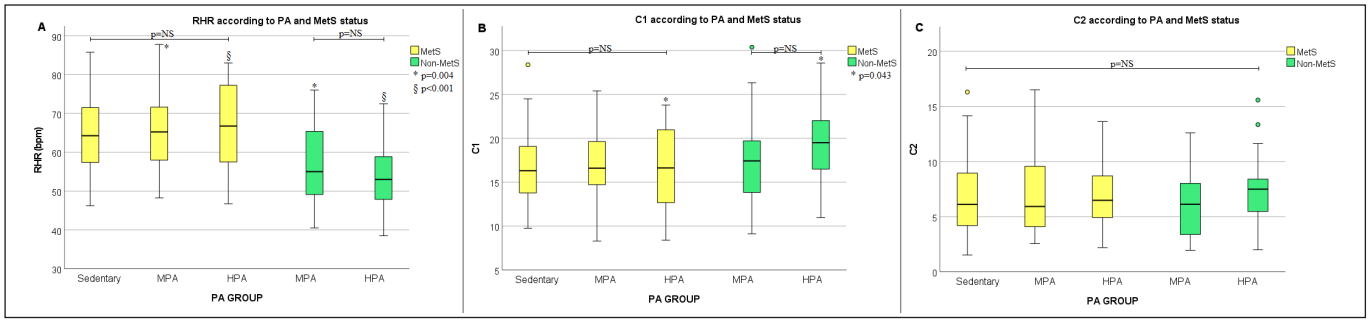
Non-MetS subjects had lower RHR in both activity groups compared to subjects with MetS: 56.9 ± 10.7 bpm vs 65.5 ± 9.6 bpm (p = 0.004) for MPA groups & 53.5 ± 8.5 bpm vs. 66.0 ± 10.5 bpm for HPA groups (p < 0.001) (Figure 3A).

Non-MetS subjects in the HPA group had slightly higher C1 values representing better large artery elasticity than MetS subjects in the HPA group: 19.1 ± 3.8 ml/mmHg × 10 vs. 16.6 ± 4.7 ml/mmHg × 10 (p = 0.043) (Figure 3B). There was no difference in C1 between MPA groups and there was no difference in C2 or LDL-C between all groups (Figures 2C, 3B–C). Non-MetS subjects in the MPA group had lower HbA1c than MetS subjects in the MPA group: 38.1 ± 2.1 mmol/mol vs. 46.5 ± 11.3 mmol/mol (p < 0.001). There was no difference in HbA1c between MetS and non-MetS subjects in the HPA groups: 43.3 ± 14.4 mmol/mol vs. 38.0 ± 3.3 mmol/mol (p = NS) (Figure 2E).

Figure 2 Laboratory results according to metabolic and physical activity status.

MetS: Metabolic syndrome MPA: Moderate physical activity group HPA: High physical activity group hs-CRP: High-sensitivity C-reactive protein HDL-C: High-density lipoprotein cholesterol LDL: Low-density lipoprotein cholesterol TG: Triglycerides HbA1c: Glycated hemoglobin.





When MetS subjects in the HPA group were compared with non-MetS subjects in the MPA group, the results of hs-CRP, HDL-C, TG and RHR were more favourable in non-MetS than MetS subjects (Table 2).

Figure 3 Resting heart rate and arterial elasticity according to metabolic and physical activity status.

MetS: Metabolic syndrome MPA: Moderate physical activity group HPA: High physical activity group RHR: Resting heart rate C1: Capacitive elasticity of large arteries C2: Reflective elasticity of small arteries.

DISCUSSION

Our main finding is that men with MetS had higher hs-CRP, TG and RHR and lower HDL-C than non-MetS individuals who had the same self-reported level of daily PA. However, there was no difference in LDL-C concentrations or small artery elasticity. The amount of PA had beneficial effects on large artery elasticity among non-Mets subjects but the same effect was not seen in subjects with MetS. The difference regarding hs-CRP, TG, RHR and HDL-C was still seen when MetS subjects with high level of daily PA were compared to non-MetS subjects with moderate level of daily PA. In the light of these results, it seems that PA alone cannot compensate for all the unfavorable cardiovascular risk factors related to MetS.

	METS HPA	NON-METS MPA	P-VALUE
hs-CRP (mg/L)	1.1 (2.2)	0.8 (0.5)	0.003
HDL-C (mmol/L)	1.2 ± 0.2	1.6 ± 0.3	0.003
LDL-C (mmol(L)	3.2 ± 1.1	3.3 ± 0.8	NS
TG (mmol/L)	2.9 ± 3.2	1.1 ± 0.4	<0.001
RHR (bpm)	66.0 ± 10.5	57.9 ± 10.7	0.009
C1 (ml/mmHg × 10)	16.6 ± 4.7	17.6 ± 5.4	NS
C2 (ml/mmHg × 100)	6.6 ± 3.0	6.3 ± 3.1	NS
HbA1c (mmol/mol)	43.3 ± 14.4	38.1 ± 2.1	NS

Table 2 Comparison of MetS subjects in the HPA group vs. non-MetS subjects in the MPA group

MPA: Moderate physical activity group HPA: High physical activity group hs-CRP: High-sensitivity C-reactive protein HDL-C: High-density lipoprotein cholesterol LDL: Low-density lipoprotein cholesterol TG: Triglycerides RHR: Resting heart rate C1: capacitive elasticity of large arteries C2: reflective elasticity of small arteries HbA1c: Glycated hemoglobin.

Subjects with MetS had higher hs-CRP than non-MetS subjects in both PA categories. Other factors such as smoking also increase the level of low grade inflammation (Tibuakuu et al., 2017). There were a few more current smokers in the MetS groups compared to the non-MetS groups. On the other hand, more subjects used statins in the MetS groups and statins have been shown to have anti-inflammatory and thus CRP lowering effects (Ridker et al., 2001). A systematic review of randomized clinical trials (RCTs) about the effects of PA on inflammation in patients with type 2 diabetes mellitus did not find any evidence that PA improved inflammation markers, but comparison was hampered because of lack of data on the intensity of exercise in some of the reviewed RCTs (Melo et al., 2017).

A meta-analysis of the effects of exercise training in patients with MetS reported small improvements in TG and LDL-C in case of aerobic exercise training but not when aerobic training was combined with resistance training which elicited only small changes on HDL-C (Ostman et al., 2017). In our study, the amount of PA seemed not to associate with the concentration of LDL-C, neither was there any difference in LDL-C between groups with different MetS status. This might be a result of the fact that physicians recognize the elevated risk for CVD related to MetS resulting in more aggressive statin treatment as using statins was more common in the MetS group. Also, we didn't separate aerobic and resistance training as all calorie expenditure was considered same.

RHR has emerged as a somewhat novel cardiovascular risk factor (Böhm et al., 2015). Especially endurance training lowers RHR among both sexes (Reimers, Knapp & Reimers, 2018), but among middle-aged to old subjects the magnitude of change is smaller than among young adults (Sandercock, Bromley & Brodie, 2005). Thi could attribute to reduced trainability of the heart muscle with advancing age. A recent small RCT among approximately 45-year-old men with high normal BP found that a 1-year isometric exercise training intervention reduced RHR without significant changes in cardiac output (O'Driscoll et al., 2022). In our study there was no change in RHR with higher activity levels in 30- to 65-year-old males with or without MetS. A possible explanation is that the PA interventions in RCTs are usually supervised and tailored to achieve certain target whereas the subjects in our study practiced PA at their own typical level, frequency and intensity. Also, the reduction of RHR could be associated to weight loss and in our study, there was no difference in BMI in groups with different levels of PA. In our study the non-MeS subjects had lower RHR than MetS subjects even though about 1/3 were using beta-blockers in the MetS group but only one (1.5%) in the non-MetS group.

Our study showed no difference in small artery elasticity between MetS and non-MetS individuals with different amount of PA. This finding is in line with an earlier study which found no difference in arterial elasticity between active and sedentary premenopausal women (Fjeldstad & Bemben, 2007). On the other hand, another study showed that endurance-trained elderly men have been found to have less arterial stiffness than their sedentary age peers (Vaitkevicius et al., 1993). We found that non-MetS subjects with high amount of PA had better large artery elasticity than their peers with MetS. Further, our finding that MetS group with high amount of daily PA had similar large artery elasticity when compared to non-MetS subjects with moderate daily PA could suggest that even though physically active lifestyle might help to preserve the elasticity of large arteries it is not alone capable of preventing the decline of arterial elasticity related to multiple cardiovascular risk factors of MetS. Our results are in accordance with a similar cross-sectional study utilizing self-reported PA among men over 60 years showing no difference in arterial elasticity between the different PA groups (Shibata et al., 2018).

An interesting result in our study was that among MetS groups the subjects with high amount of PA had lower HbA1c than those with moderate or low amount of PA, although statistically significant correlation could not be found. PA has been shown to improve insulin sensitivity (Thomas, Elliott & Naughton, 2006; Lin et al., 2015) and in our study the MetS subjects with high amount of PA had the same level of HbA1c as the non-MetS subjects despite higher body mass index (BMI). Similar differences in HbA1c were not seen in non-MetS subjects with different amount of PA which could mean that PA is capable of improving impaired glycemic control but the effects are minor in case of normal glycemic status. This is supported by recent study evaluating the effects of 15-week exercise programme in 50–70-year-olds with low baseline fitness as there was no effect on Hba1c but only 7% of the study subjects had DM (Nielsen et al., 2021). It is also possible that the findings could be a result of less subjects with abnormal fasting glucose in the HPA group of MetS subjects as the NCEP definition of MetS enables diagnosis of MetS with three other components even in the case of normal fasting glucose. We didn't specify the MetS components of each subject but there was no difference in the BMI of MetS groups and there was no difference in the rate of subjects using glucose lowering drugs in the MPA and HPA MetS groups.

The strength of this study is that we were able to test the effects of PA on several different CVD risk factors such as arterial stiffness and RHR which are infrequently under research. The subjects were 30–65-year-old men, which is a favorable patient group for lifestyle interventions regarding both primary and secondary prevention of CVD. Even regular small tasks such as practicing recreational football for 50 minutes once a week has been found to improve some CVD risk factors among 35–55-year-old sedentary men (Modena et al., 2022). The key limitation of this study is that we relied purely on the subjects' self-perceived assessment of their PA instead of monitoring their PA with modern activity devices. Although we used a structured questionnaire based on earlier studies (Aittasalo, Miilunpalo & Suni, 2004; Ainsworth et al., 2011), we didn't utilize any validated questionnaires such as International Physical Activity Questionnaire (IPAQ) (Craig et al., 2003). This corresponds to the typical outpatient or acute clinical scenario where a physician has to rely on the patient's own assessment of his physical activity. Objective data could nowadays be collected with fitness trackers, accelerometers or smart watches at reasonable cost to complement the subjective assessment, but it has been suggested that self-report questionnaires shouldn't be completely replaced by consumer fitness trackers in research purposes as the sensors in the devices measure motion in a specific way so all steps taken or types of sport are not recognized similarly (Grossi, Gattringer & Batinic, 2021).

Another limitation regarding the self-reported PA is that our questionnaire utilized the subjects' self-perceived intensity of the PA at hand: two persons can obviously have a very different view regarding the intensity of a certain activity, usually depending on the physical condition and the sports history they have themselves. Taking into consideration the substantially higher BMI of the MetS subjects there's a high probability that MetS subjects were less accustomed to physical exercise. Poor physical condition will lead to a perception of more vigorous exercise than a person with better physical fitness might experience during similar activity. This could have resulted in overestimation of PA in the MetS group. Although we used international compendium of estimations for MET-values this might be one of the reasons why MetS subjects with higher amount of PA didn't overtake non-MetS subjects with lower amount of PA. Obese subjects have been found to both underreport their energy intake and overestimate their daily PA (Lichtman et al., 2010). We also didn't have enough sedentary non-MetS subjects for analysis.

CONCLUSIONS

Men with MetS and high amount of daily PA had still higher RHR, hs-CRP, TG and lower HDL-C than their non-MetS counterparts who had moderate level of self-reported daily PA. There was no difference in blood lipids, hs-CRP, RHR or arterial elasticity among men with MetS and different amounts of self-reported daily PA. Based on the findings of this study, increasing PA alone is an insufficient strategy for improving all the unfavorable CVD risk factors related to MetS. Future research should explore the effects of combined lifestyle changes such as weight loss together with PA on CVD risk factors among men with MetS.

DATA ACCESSIBILITY STATEMENT

The data used and/or analysed during this study are available from the corresponding author on reasonable request.

ABBREVIATIONS

BMI: body mass index

BP: blood pressure

C1: capacitive elasticity of large arteries

C2: reflective elasticity of small arteries

CVD: cardiovascular disease

DM: diabetes mellitus

HbA1c: glycated hemoglobin

HDL-C: high-density lipoprotein cholesterol

hs-CRP: high-sensitivity C-reactive protein

IPAQ: international physical activity questionnaire

IQR: interquartile range

LDL-C: low-density lipoprotein cholesterol

MET: metabolic equivalent of task

MetS: metabolic syndrome

NCEP: National Cholesterol Education Program

PA: physical activity

RCT: randomized clinical trial

RHR: resting heart rate

TG: triglycerides

ETHICS AND CONSENT

The study followed the ethical principles of the Declaration of Helsinki and each study subject gave a written informed consent. The study protocol was approved by the Research Ethics Board of Kanta-Häme Hospital District.

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COMPETING INTERESTS

The authors have no competing interests to declare.

TRIAL REGISTRATION

This study is registered at www.clinicaltrials.gov (No. NCT01119404).

AUTHOR CONTRIBUTIONS

AP designed the study. HS, HPM and AP participated in the acquisition of data, analysis and drafting of the manuscript. JL and RA helped in drafting the manuscript. All authors read and approved the final manuscript.

AUTHOR AFFILIATIONS

Harri J. Saarinen orcid.org/0000-0002-9807-6681
Tampere Heart Hospital, FI

Hanna Pohjantähti-Maaroos orcid.org/0000-0002-8767-239X
Tampere Heart Hospital, FI

Riitta Antikainen orcid.org/0000-0001-5262-362X
Center for Life Course Health Research, University of Oulu, FI

Jorma T. Lahtela orcid.org/0000-0003-0752-8089
Tampere University Hospital, FI

Ari Palomäki orcid.org/0000-0001-9759-4332
Kanta-Häme Central Hospital, FI

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