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Title**: ASSESSMENT OF CARDIOVASCULAR RISK AND VASCULAR AGE IN OVERWEIGHT/OBESE ADULTS WITH PRIMARY HYPERTENSION: EXERDIET-HTA STUDY**

Running title**: Cardiovascular risk and hypertension**

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Abstract

Objectives: Hypertension (HTN), obesity and low cardiorespiratory fitness (CRF) are associated with an increased risk of a cardiovascular event. Taking part overweight/obese individuals with HTN the aims of the current study were: to estimate cardiovascular risk (CVR) and vascular age (VA) profiles analyzing potential sex differences; to determine whether VA is higher than chronological age (CA) and, whether CVR is associated with a low level of CRF.

Methods: Overweight/obese non-Hispanic white participants (n=209; 141 men and 68 women) with primary HTN had their CVR and VA determined using the New Pooled Cohort Risk Equations and The Framingham method, respectively. Considering values of peak oxygen uptake, participants were divided into tertiles for each sex.

Results: The CVR, but not VA (p=0.339), was higher (p<0.001) in men compared to women irrespective of age. Irrespective of sex VA was higher than CA (p<0.001). Age and body mass index were higher (p<0.05) in the low CRF group compared to other groups. There were no differences in CVR (p=0.907) and VA (p=1.643) when values were separated into CRF groups.

Conclusions: Pooled Cohort Equations could underestimate the risk of suffering a cardiovascular event in the following 10 years in overweight/obese non-Hispanic white women with HTN compared to men. The VA appears to be a useful tool in communicating CVR in this population irrespective of sex. The CRF alone may not be enough to moderate the CVR.

Trial Registration: NCT02283047

Keywords: Cardiovascular disease; Chronological age, Cardio-respiratory fitness.

Introduction

Cardiovascular disease (CVD) is the leading cause of early morbidity and hospitalization in the world [1]. According to World Health Organization 17.5 million people died from CVD in 2012, representing 31% of all deaths, of which 7.4 and 6.7 million were due to coronary heart disease and cardiovascular (CV) events, respectively [2]. Furthermore, a large number of individuals have a heightened risk of CVD because they have two or more associated risk factors [1,3,4].

Several attempts have been made to determine CV risk (CVR) factors associated with having a cardiac event. Guidelines indicate that age, sex, diabetes, smoking, cholesterol and hypertension (HTN) have a causal relationship with CV events and premature death [1,3,5]. High blood pressure (BP), often referred to as HTN, is the most common CVR factor which leads to heart failure, stroke, angina and premature death if not detected early and treated adequately [6]. Additionally, population based research suggest that obesity is directly related to HTN [7]. An appreciation of the clinical significance of obesity-related HTN has since grown substantially over time. As such, obesity is now recognized as a major cause of HTN (at least 75% of all cases) and the combination of both are well known to increase CVR [7]. Additionally, CVR factors, associated with the development of CVD, are similar in both sexes [8]. However, with the same risk factors, CVR is two to five times more common in men than in women, *i.e*. women have a lower predicted 10-year risk [9]. It has previously been suggested that this discrepancy may be due to the protective action of estrogens [8] or because substantial disparities exist in the prevention, recognition, management and clinical outcomes of CVD in women [10]. A CVR score can be used to determine CVD using the Pooled Cohort Equations and the Framingham Heart Study which are tools commonly used to define the 10-year risk of developing CVD [9]. An additional tool for evaluating the overall CVR is vascular age (VA). This is a quite novel concept derived from Framingham CVR tables, which indicates the biological age of an individual's arteries (*i.e.,* the age of the vascular system of a person with different

CVR factors) [1,11]. This process is accelerated with the presence of additional CVR factors and is associated with changes in the mechanical and the structural properties of the vascular wall, which leads to poor endothelial health and the loss of arterial elasticity [1,7].

The relationship between poor lifestyle and increased CVR is well documented. As such there is a persistent need to discuss cardio metabolic lifestyle factors with all patients, in order to reduce CVD, and control the CVR factors. Cardiovascular risk factors can be classified as modifiable and non-modifiable. Non-modifiable risk factors are (but not limited to) age, sex and family history. Modifiable CVR factors are cardio metabolic lifestyle factors and can be altered during the cause of one life; these include but are not limited to smoking, alcohol, diet and importantly physical activity [12]. Higher levels of physical activity can improve the CVR in diseased or at risk patients. Cardio-respiratory fitness (CRF) can be used to quantify these positive effects as it is negatively associated with a reduction in CV morbidity and mortality [12].

Currently, there is no known research that measures CVR and VA and its association with CRF in primary hypertensive and overweight/obese adults. Considering the importance of CVR factors and the limited scientific literature, the aims of the study were: 1) to estimate CVR and VA profiles of overweight/obese patients with HTN, analyzing potential sex differences, (2) to determine whether VA is higher than chronological age (CA) and, (3) to determine whether CVR is associated with a low level of CRF.

Methods

Study design

Baseline data from EXERDIET-HTA randomized controlled experimental trial were taken for the purpose of this study [13]. The design, selection criteria and procedures for the EXERDIET-HTA study have been previously detailed [13]. The study protocol was approved by The Ethics Committee of The University of the Basque Country (UPV/EHU, CEISH/279/2014) and the

Ethics Committee of Clinical Investigation of Araba University Hospital (2015-030), and all participants provided written informed consent prior to any data collection.

Participants

Non-Hispanic white participants (n=209) with primary HTN (≥140 Systolic BP (SBP) and ≥90 diastolic BP) [4], who were classified as being overweight (body mass index, BMI≥25) or obese (BMI≥30) [14] were recruited from the cardiology services and local media. .

Cardio-respiratory fitness

Cardio-respiratory fitness was determined using a cardiopulmonary exercise test to assess $\dot{V}O_{2peak}$. Briefly, the test was performed on an electronically braked Lode Excalibur Sport Cycle Ergometer (Groningen, The Netherlands). The testing protocol was started at 40W (70 rpm) with gradual increments of 10W every minute until exhaustion with continuous electrocardiogram monitoring. Expired gas analysis was determined using a commercially available metabolic cart (Ergo CardMedi-soft S.S, Belgium Ref. USM001 V1.0) which was calibrated before each test with a standard gas of known concentration and volume. Breath by-breath data were measured continuously during exercise before being averaged over each 60 second periods. Blood pressure was measured every two minutes throughout the test. At the end of each stage the rate of perceived exertion (6 to 20) was recorded (Borg Scale). Peak oxygen uptake was defined as the highest oxygen uptake value attained toward the end of the test. Achievement of $\dot{V}O_{2peak}$ was assumed with the presence of two or more of the following criteria: 1) volitional fatigue (>18 on Borg scale), 2) peak respiratory exchange ratio ($\dot{V}CO_2/\dot{V}O_2$) ≥1.1, 3) achieving >85% of age predicted maximum heart rate, and 4) failure of $\dot{V}O_2$ and/or heart rate to increase with further increases in work rate [13].

The distributions of $\dot{V}O_{2peak}$ were divided into tertiles for each sex: The lowest tertile (Low-CRF group); $\dot{V}O_{2peak}$ ≤21 in men and $\dot{V}O_{2peak}$ ≤16 in women; the intermediate tertile (Medium-CRF

group); 21.1<$\dot{V}O_{2peak}$ ≤26 in men and 16.1<$\dot{V}O_{2peak}$≤21 in women and the highest tertile (High-CRF group); $\dot{V}O_{2peak}$>26 in men and $\dot{V}O_{2peak}$>21 mL·kg·min^{-1} in women.

Measurement of CVD Risk Factors

Established CVR factors used in the present study to determine the CVR and VA of participants were defined as follows:

Blood pressure. Ambulatory BP monitoring was conducted over a 24 hour period using an oscillometric ABPM 6100 recorder (Welch Allyn, New York, USA) to evaluate SBP (as used to determine CVR) [9]. The device was used in line with the recommendations set by the ESH/ESC guidelines. As such BP was measured at 30-minute intervals during the awake-time and at 60-minute intervals during periods of sleep. Data were only used if at least 75% of the awake-time and sleep periods were successfully recorded [13].

Blood sampling. Fasting venous blood (12.5ml) was obtained from each participant. Diabetes mellitus (DM) was defined as a fasting glucose of ≥126 mg/dL. Additionally, measurements of glucose and lipid profile (total-, HDL- and LDL-cholesterol) were assayed.

Self report. Age and cigarette smoking status were assessed by self-report. All medicines were ascertained from the participant's physician.

Cardiovascular risk. The Framingham Heart Study is a quantitative method used in primary care for assessment of general CVR profile. The absolute risk applies to the individual, a score of 10% means that there is a 10% chance of having a CV event within the next 10 years [9,11]. Under 6% is considered low risk; between 6 and 20% medium risk, and ≥20% high risk [11]. Recently, New Pooled Cohort Risk Equations have been developed from the Framingham Heart Study [9,11]. The equation to estimate the 10-year risk of developing a first atherosclerotic CVD event were developed from sex- and race-specific proportional-hazard models that included the covariates of age, treated or untreated high SBP level, total cholesterol and HDL-C concentrations, current smoking status, and history of DM. For the equation, the values for

age, lipids, and SBP were log transformed. Interactions between age and lipids or age and SBP used the natural log of each variable. Calculation of the 10-year risk estimate for hard atherosclerotic CVD is described as a series of steps [9].

Vascular age. The Framingham method was used to determine the VA of all participants [11]. The VA indicates the biological age of the individual's vascular system, as the age a person would be with the same calculated CVR but whose risk factors were all within normal ranges. The sex specific risk factors considered were age, HDL-C, total cholesterol, SBP, DM and smoking status. Each variable received a weighted score; the sum of the score for each variable was then translated to the risk of a CV event in 10 years and the VA [11].

Statistical analysis

Descriptive statistics were calculated for all variables. Data are expressed as mean±standard deviations (SD). All variables were deemed normally distributed using a Kolmogorov-Smirnov apart from, age, total cholesterol, HDL-C, CVR and VA which had a skewed distribution and were therefore log transformed prior to any analysis.

A 2 sample *t*-test was used to determine whether there was a significant sex difference for the variables: age, BMI, SBP, total cholesterol, HDL-C, antihypertensive medication, cigarette smoking and DM. Analysis of covariance (ANCOVA) was used to examine the dependant variables (age, BMI, SBP, total cholesterol, HDL-C, antihypertensive medication, cigarette smoking, DM, CVR and VA) of the participants classified by CRF level (low, medium and high). A Bonferroni test was used to determine the level of significance when a significant main effect was found. Statistical significance was set at $p<0.05$. The statistical analyses were performed with the SPSS version 22.0 software package.

Results

The characteristics of CVR factors classified by sex are presented in Table 1. The mean age (±SD) of participants was 54.0±8.1 yrs with 67.5% and 32.5% being men and women,

respectively. All participants were classified as obese (BMI>30 kg/m^2) in accordance with AHA/ACC/TOS guidelines for the Management of Overweight and Obesity in Adults [14]. Although 87.1% of all participants were taking anti-hypertensive medication, mean SBP suggested that all participants irrespective of sex were pre-hypertensive. However, there was a trend to suggest that women had a lower SBP compared to men (mean difference=1.6; 95%, confidence interval (CI)=-2.5-5.7 mmHg). Mean total cholesterol was similar in men and women with both sexes exceeding cut-off values set by the ESH/ESC guidelines [4], but there was also a trend to suggest that women had lower total cholesterol than men (mean difference=3.3; 95% CI=-30.6-37.3 mmHg). Moreover, HDL-C was significantly higher (p=0.002) in women than men (mean difference=5.0, 95% CI=-13.3-3.3 mg/dL), but both were inside the healthy cut-off values suggested by the ESH/ESC guidelines [4]. Smoking was present 11.4% of the participants and 4.5% of the sample was suffering from DM.

The absolute CVR was significantly different (p<0.001) between sexes with women having a lower CVR than men (mean difference 5.8, 95% CI=-3.8-7.7 %, Table 1). Additionally, in accordance with the Framingham study and ACC/AHA Guideline on the Assessment of CVR [9,11] men were considered to be at medium risk whereas women were considered low risk. As shown in Table 2, there were no sex differences in VA (mean difference=1.4, 95% CI=-2.4-5.1 yrs). However, VA was significantly higher (p=0.001) than CA (mean difference=8.8, 95% CI=7.2-10.3 yrs).

Characteristics of the participants separated into CRF groups are presented in Table 3. Bonferroni analysis revealed that age was higher (p<0.05, Δ=-9.7%) in the low CRF group compared to the high CRF group. Moreover, BMI was higher in the low CRF group compared to the medium CRF group (34.0±0.5 *vs.* 31.2±0.5 kg/m^2; mean difference=2.8, 95% CI=1.1-4.4 kg/m^2) and to the high CRF group (34.0±0.5 *vs.* 28.5±0.5 kg/m^2; mean difference=5.5, 95% CI=3.8-7.2 kg/m^2). No significant differences were observed among CRF groups for SBP, total cholesterol, antihypertensive medication, smoking, DM or HDL-C (p>0.05).

Bonferroni analysis revealed that no significant differences were observed in CVR (p=0.907) and VA (p=1.643), when values were separated into CRF groups (Table 3). However, the low CRF group showed an upward trend in VA with higher values than those with medium CRF group (mean difference=0.5, 95% CI=-4.1-5.0 yrs) or higher CRF group (mean difference=3.4, 95% CI=-1.5-8.4 yrs).

Discussion

To our knowledge this is the first known study to estimate the CVR and VA in overweight/obese people with HTN and its association with CRF level using the recently developed Pooled Cohort Equations [9] and the original 2008 Framingham model. The main findings of this study were: 1) CVR is significantly higher in men than in women despite them having same CVR values and this is not effected by age; 2) predicted VA is significantly higher than CA in overweight/obese people with primary HTN with no sex differences, and 3) CRF alone did not appear to significantly moderate CVD. These findings highlight the importance of being able to determine the CVR and VA of overweight/obese people with primary HTN. Considering the known increase in CVD in hypertensive patients and the relative ease of predicting 10-year CVR; defining CVR and VA in a hypertensive population would likely be a useful tool for clinicians.

Although the role of major CVR factors in the development of CVD and VA were similar in both sexes, Table 1 shows that CVR is 57% higher in men than in women (p=0.001), with an estimated 10-year risk of hard atherosclerotic CVD event of 10% in men and 4.3% in women, which represents medium and low risk, respectively. As such, it is likely that men have a higher risk of suffering a CV event in the following 10 years. The difference in the HDL-C level could be one of the determinants of the sex difference in CVR profile in the present study, with women showing higher concentrations compared to men. It is well known that high concentrations of HDL-C prevent the development of atherosclerosis and CVD. In particular, the transport of

reserve cholesterol and the inhibition of oxidized LDL-induced monocyte infiltration can avoid this development [15]. In addition, the Framingham Heart Study showed that HDL-C was the most powerful lipid predictor of CHD risk in both sexes >49 yr old. For every 1mg/dL increment in HDL-C, there was an associated 2% decreased in the risk of CHD in men, and a 3% decrease in women [16]. Previous studies have shown that the increased CVR in men was hidden behind an almost-normal classic lipid profile, being total cholesterol, LDL-C, and TG higher, and HDL-C concentrations significantly lower than women (pre or post menopausal status), with all values within the normal range [17]. In our study, both sexes presented hyperlipidemia values (total cholesterol>190mg/dL) and prehypertensive SBP values with slight, but concomitant variation (*i.e.,* an upward trend in men compared to women), which has been shown to increase the risk of coronary heart disease and promotes a poor cardio metabolic profile [17]. It seems that there is a cardio protective effect of endogenous estrogens in premenopausal women, when compared with age-matched men, due to an enhanced HDL quality. However, in postmenopausal women there is a trend to present with a significantly increased BMI, BP, LDL-C and total cholesterol, along with a reduction of absolute HDL-C concentration, with changes in the composition of HDL particles [17,18]. In the present study, 51.5% of women were premenopausal state. Therefore, it should take into account that in the current study: 1) both men and women were in normal HDL-C values (>40 mg/dL for men, and >46 mg/dL for women) [4], along with obesity, hyperlipidemia and primary HTN diagnostic, 2) almost half of the women were on postmenopausal state and 3) the sex-specific risk prediction model used in the Pooled Cohort Equation always calculates a higher risk in men compared to women, despite them having same CVR values and regardless of age [9]. As such, women could show an underestimated CVR profile with less aggressive treatment strategies. Nowadays, a greater number of women die annually from CVD compared with men [19]. Thus, although sex differences are well documented in the prevalence of the CVR factors, the clinical manifestation and incidence of CVD and the impact of risk factors on outcomes [20] in women

is often underestimated due to the misperception that females are “protected” against CVD [21].

As expected, VA was significantly higher (p=0.001) than CA (Table 2) (VA=62 *vs.* CA=54 yrs). As such, people with primary HTN and obesity are “older” for their VA when compared with their CA. It is, therefore, likely that the health of the heart and blood vessels are more deteriorated than they should be in this population [11]. There is good evidence that CA is strongly and independently related to CVR, but this is not necessarily so for the VA. Repeated exposure to CVR factors across the lifespan leads to between-subject differences in VA and CA [22]. Previous research has shown that in apparently healthy and asymptomatic people, 72.45% of the individuals had a greater VA than the CA, suggesting that this risk may be underestimated [23]. The current study suggests that key contributors of an increased VA in a hypertensive population are dyslipidemia, SBP, treatment for HTN, obesity and smoking (87.2% of them showing a greater VA than CA). Thus, VA seems to be a useful tool in communicating about risk to individuals, such as hypertensive and obese patients, who are at a greater risk of a CVD event, and warrant early intervention of modifiable CVR factors (*i.e.,* healthy eating, increasing physical activity, cessation of cigarette smoking and alcohol).

It is well recognized that physical activity is an important factor for reducing CVR and CVD [9,12]. Previous research has suggested that people with sedentary behaviors are at a higher risk of all-cause mortality compared with those who do at least 150 minutes of moderate-intensity, or 75 minutes of vigorous-intensity aerobic physical activity per week, as recommended by WHO [12]. In the current study it was found that CRF alone does not significantly moderate CVR, due to no differences among three CRF groups (Table 3). However, mean difference and CI suggest that those who are in the low CRF group, have meaningfully higher VA (although no significant) (Table 3); which suggests that CVR may in-part be moderated by CRF but in conjunction with other CVR factors such as BMI and age. Aging and inactivity are associated with a decrease in both CRF and morphological changes in all layers of

the vascular tree, and these are accompanied by increased arterial stiffness and aortic pressure, leading to a higher VA [24]. Furthermore, in the present study BMI explained the 22.5% and age 6% of the variance between the CRF groups. The current study reinforces the evidence linking obesity, age and CRF level with VA in primary hypertensive population [7,25], suggesting that low CRF could potentially be used in conjunction with other CVR factors to help determine predisposal of CVD, but not as a factor in its own right. Previous studies have concluded that physical activity is associated with beneficial changes in circulating lipids and lipoproteins, body weight, BP and insulin sensitivity, having a significant reduction in CVR [12,25]. In line with that, the last 2016 European Guidelines on CVD prevention in clinical practice highlight the CRF as a factor that might influence the relationship between adiposity and clinical prognosis in the "obesity paradox". Thus, normal weight individuals with a low CRF have a higher risk of mortality than fit individuals, regardless of their BMI [1,26].

Although our study has highlighted the importance of determining CVR factors in a hypertensive population, several limitations should be acknowledged. Firstly, although our sample size was sufficient as an initial investigation into CVR and HTN; it would not be comparable with that of larger epidemiological studies and future studies should consider large scale investigations as well as determining the short and long-term effects of different interventions. Secondly, the current study only had 32.5% of women which does not represent an equal gender split. As this poses statistical issues, future studies, particularly those using interventions should look to recruit equal numbers. Lastly, although CRF did not sufficiently moderate CVR in the current study, the effects of physical activity in a hypertensive patients with a broader range of $\dot{V}O_{2peak}$ should be further investigated in conjunction with other modifiable risk factors to determine the effectiveness of moderating CVR and VA. Additionally, future studies should look to determine whether VA and CVR can be used to more easily inform patients of their cardiovascular health than current methods.

Conclusions

Findings suggest that male non-Hispanic white overweight/obese individuals with diagnosed HTN have a higher risk of suffering a CV event in the following 10 years compared to females according to the greater CVR assessed using the Pooled Cohort Equations. However, CVR in women could be underestimated when both sexes present with the same CVR values and age. Those who are overweight/obese with HTN have an "older" VA compared to their CA irrespective of sex. Finally, although CRF alone did not moderate CVR in the current study, further research into the effect of physical activity alongside additional modifiable CVR factors in HTN populations, particularly those who are the least active is warranted. Predictions of CVR and VA estimation may represent as a useful clinical tool for detecting patients at risk of a cardiac event, but estimation equations should be more focused on sex related differences.

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