Maturitas 76 (2013) 57–63

Contents lists available at SciVerse ScienceDirect

Maturitas

journal homepage: www.elsevier.com/locate/maturitas

Effects of interactions between ApoE polymorphisms, alcohol consumption and obesity on age-related trends of blood pressure levels in postmenopausal women: The Bambuì cohort study of aging (1997–2008)

Maria Léa Correa Leite[a,*], Emilio H. Moriguchi[b], Maria Fernanda Lima-Costa[c]

[a] Institute of Biomedical Technologies, National Research Council, Milan, Italy
[b] Federal University of Rio Grande do Sul, University of Vale dos Sinos, Moinho de Vento Hospital, Rua Vicente da Fontoura 2719, 90640-003 Porto Alegre, RS, Brazil
[c] Oswaldo Cruz Foundation, Rene Rachou Research Institute, Federal University of Minas Gerais Medical School, Av. Augusto de Lima 1715, 30190-002 Belo Horizonte, MG, Brazil

ARTICLE INFO

Article history:
Received 26 February 2013
Received in revised form 30 April 2013
Accepted 20 May 2013

Keywords:
Alcohol consumption
Apolipoprotein E
Blood pressure
Obesity
Menopause

ABSTRACT

Objectives: To evaluate the effects of interactions between ApoE genotypes, alcohol consumption and obesity on the age-related trends of blood pressure (BP) levels in postmenopausal women.
Study design: A population-based prospective cohort study of all residents in Bambuì, south-eastern Brazil, aged 60 years or older. Repeated BP measurements were obtained in four waves from 851 women who underwent ApoE genotyping at baseline (88.3% of those enrolled), and multi-level random-effects pattern-mixture models were used to evaluate the age-related BP trajectories, while accounting for non-ignorable dropouts/deaths and handling heterogeneities as random parameter variations. The few measurements (2.1%) made during hormone replacement therapy were excluded from the analysis.
Results: Alcohol consumption was associated with high levels of systolic and diastolic BP in an age × genotype-dependent manner only in the non-obese women (BMI < 27 kg/m^2). Among those with the ε3/3 genotype, the differences in systolic and diastolic levels between drinkers and non-drinkers estimated at the age of 60 years were respectively 13.7 mmHg ($p = 0.022$) and 10.7 mmHg ($p = 0.002$), and disappeared in the older age groups, in which drinking was associated with systolic/diastolic hypertension if the non-obese women were ε4 carriers.
Conclusion: In non-obese postmenopausal women, alcohol consumption is associated with systolic and diastolic hypertension early in those with the ε3/3 ApoE genotype, and late in ε4 carriers. We hypothesize the mediation of androgen hormones and the influence of ApoE genotypes on age at natural menopause. A better understanding of these mechanisms may guide better preventive choices.

1. Introduction

Blood pressure (BP) generally increases after menopause, and hypertension is a major risk factor for cardiovascular disease (CVD), the leading cause of death in postmenopausal women [1].

Heavy alcohol consumption has been consistently associated with a higher risk of hypertension in women [2], but the data concerning the effect of light to moderate alcohol consumption are conflicting [3–5]. Light alcohol consumption seems to have a protective effect on hypertension in pre-, but not postmenopausal women [6,7].

The apolipoprotein E (ApoE) gene encodes a plasma protein that plays an important role in transporting cholesterol, and the effects of its polymorphism on lipids and the risk of CVD have been extensively studied [8]. The ApoE gene has three common alleles (ε2, ε3 and ε4) and six different genotypes (ε2/2, ε2/3, ε2/4, ε3/3, ε3/4, and ε4/4). It is believed that allele ε3 codes for normal ApoE functions, whereas alleles ε2 and ε4 have a definite impact on lipids and lipoproteins: ε2 tends to be associated with increased triglyceride levels and decreased levels of total and low density lipoprotein (LDL) cholesterol, and ε4 is associated with increased total and LDL cholesterol levels [9].

It is still not clear whether ApoE polymorphism plays a role in regulating BP, and the conflicting findings of studies of the relationship between ApoE polymorphism and BP have aroused interest in the interactions involving this polymorphism and lifestyle variables [10].

The Bambuì Cohort Study of Aging is an ongoing prospective study of an elderly Brazilian cohort; we used its database to examine the individual longitudinal trajectories of BP levels in order

* Corresponding author at: Institute of Biomedical Technologies/CNR, Via Fratelli Cervi 93, 20090 Segrate, MI, Italy. Tel.: +39 0226422730; fax: +39 0226422770.
E-mail addresses: lea.correa@itb.cnr.it (M.L. Correa Leite), moriguch@terra.com.br (E.H. Moriguchi), lima-costa@cpqrr.fiocruz.br (M.F. Lima-Costa).

0378-5122/$ – see front matter © 2013 Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.maturitas.2013.05.012

to assess the interactions between ApoE genotypes and lifestyle factors. In a previous paper, we described the results relating to men and showed the effect of an interaction between allele ε2 and alcohol consumption on the age-related trends of BP levels [11]. Similar findings have been reported in the case of middle-aged men, but not middle-aged women [12]. However, there is no published information concerning postmenopausal women.

The aim of this study was to evaluate the interactions between ApoE genotypes and alcohol consumption in elderly women and, as it has been shown that body weight affects the association between alcohol drinking and BP levels in women [13], we also examined the role of the body mass index (BMI).

2. Methods

2.1. Subjects and measurements

The Bambuí Cohort Study of Aging was carried out in Bambuí, a city of approximately 15,000 inhabitants in south-eastern Brazil, and its procedures have been described in detail elsewhere [14]. Briefly, the baseline cohort population consisted of all residents aged ≥60 years on 1 January 1997, who were identified by means of a complete census of the town. Of the 1742 older residents, 1606 (92.2%, including 964 women) participated in the baseline survey, which consisted of a questionnaire covering socio-demographic, lifestyle, medical and drug use data, laboratory tests (biochemical and hematological analyses), and physical measurements (BP and anthropometric measures). The follow-up was based on annual interviews and reviews of medical records, and collected information concerning the current use of anti-hypertensive medication and hormone replacement therapy (HRT). The Bambuí Cohort Study of Aging was approved by the Ethics Committee of the Fundação Oswaldo Cruz, Brazil, and all of the participants signed an informed consent form.

Systolic and diastolic BP were measured at baseline (1997), and after three (2000), five (2002) and eleven (2008) years, using standard desk mercurial sphygmomanometers (Welch Allyn Tycos 5097-30, Tycon, Arden, USA) and stethoscopes (Littman Cardiology II, 3 M Medical Devices, St. Paul, USA), and a standard (12 cm × 23 cm) or large adult cuff size (15 cm × 32 cm) depending on the subject's right arm circumference. At each time point, three measurements separated by 2-min intervals were made after an initial 5-min rest, and 30 min or more after the last caffeine intake or last cigarette. The measurements were made in the early morning in a quiet, isolated, temperature-controlled room at the project field clinic by appropriately trained technicians, with the subjects seated with their arm supported at heart level. The recorded BP was the arithmetic mean of the second and third measurements.

Genomic DNA for ApoE genotyping was extracted from blood samples using the Wizard® Genomic DNA Purification System (Promega, Madison, WI, USA). The DNA samples were then amplified by means of polymerase chain reaction (PCR), digested by *Hha*I, and underwent restriction fragment length polymorphism analysis as previously described [15]. The PCR involved the use of the following primers: forward 5′ TAA GCT TGG CAC GGC TGT CCA AGG A 3′ and reverse 5′ ACA GAA TTC GCC CCG GCC TGG TAC AC 3′. The PCR conditions were denaturation at 95 °C for 5 min, followed by 35 cycles of 95 °C for 1 min, 60 °C for 1 min, and 70 °C for 2 min, and a final extension at 72 °C for 10 min. The restriction fragment length polymorphism analysis yielded the following patterns: **ε2ε2**, 91 and 83 bp; **ε3ε3**, 91, 48 and 35 bp; **ε4ε4**, 72, 48 and 35 bp. Each of the heterozygote genotypes contained both sets of fragments from each ApoE allele.

The current use of antihypertensive drugs (AHDs) was ascertained annually by reviewing medication containers and/or physician prescriptions made during home visits to all of the participants. The medications were coded on the basis of the Anatomical Therapeutic Chemical (ATC) classification system [16], and were considered AHDs if they fell into ATC groups C02 (antihypertensives), C03 (diuretics), C07 (beta-blockers), C08 (calcium channel blockers), or C09 (agents acting on the renin-angiotensin system). For the purposes of this analysis, the women using any type of AHD were considered "treated". The participants were divided into four groups on the basis of the number of years on treatment in relation to the number of years of follow-up: 1 = untreated; 2 = irregularly treated (treated for less than 50% of the follow-up); 3 = frequently treated (≥50%); and 4 = regularly treated.

For the alcohol consumption estimates, the subjects were shown cards with a representation of the amount of liquid corresponding to one drink of spirits, beer or wine. Alcohol consumption was calculated by multiplying the number of drinks by the frequency of imbibing in a week during the previous 12 months. We considered two categories: "non-drinkers" were teetotallers and ex-drinkers (those who had stopped drinking at least 12 months previously), and "drinkers" were those currently drinking any amount of alcohol. The BMI was calculated as weight(kg)/height^2(m), and we considered two groups: "obese" (or highly overweight), defined as a BMI of ≥27 kg/m^2 and "non-obese". Current smokers were defined as those who had smoked at least 100 cigarettes during their lifetime and still smoked. The smoking, BMI and HRT data were updated after three, five and eleven years of follow-up.

2.2. Statistical analysis

Multi-level, random-effects pattern-mixture (MLREPM) models [17] were used to evaluate the age-related BP trajectories while accounting for non-ignorable dropouts/deaths. This approach has been described in more detail in a previous paper [18]. In brief, the variable defining three groups on the basis of the pattern of their missing data (completers, lost because of drop out, lost because of death) was included as a model covariate, and the overall estimates of the trend parameters were obtained by means of weighted averaging (mixing) the pattern-specific estimates. The weights were the estimated proportion of women in the three missing-pattern groups within each genotype × drinking × BMI group, and the standard error of the averaged overall estimates were obtained using the delta method. In a hierarchical (multi-level) structure, AHD use constituted the third level (treatment groups, subjects within treatment group and BP measurements within subjects) and, in order to avoid the problem of over-parameterisation, we addressed heterogeneity in terms of random slope parameter variations across the four treatment groups. Age at baseline was included in the models to account for birth year effects, and the individual contributions to the age-related trends were spread over a single age axis ranging from 60 to 90 years. As the inclusion of quadratic age terms did not significantly improve the likelihood of the models, only linear terms were fitted. Examination of the residual plots did not show any particular trend in their distribution, thus indicating the good fit of the models to the observed data.

The analyses were made using the multi-level mixed-effects linear regression (xtmixed) procedure of Stata software, version 11.2 [19].

3. Results

Of the 964 enrolled women, 851 (88.3%) underwent ApoE genotyping: 549 (64.4%) were ε3/3, 186 (21.9%) ε3/4, 94 (11.0%) ε2/3, 12 (1.4%) ε4/4, and 10 (1.2%) ε2/4: none was ε2/2. The allele frequencies were within Hardy–Weinberg equilibrium ($p > 0.25$). The women with the ε3/4 or ε4/4 genotype were grouped in a single ε4 carrier group, and the 10 women with the ε2/4 genotype (none

Table 1
Patterns of missingness of blood pressure measurements in the 841 women.

Pattern	Missing because of		Total
	Dropout	Death	
O O O O (Completers)			402
O O M O			16
O M O O			18
O M M O			3
Total			439
O O O M (missing after the third wave)	71	120	191
O M O M	4	7	11
O O M M (missing after the second wave)	20	61	81
O M M M (missing after the first wave)	25	94	119
Total	120	282	

O: observed; M: missing.

of whom was a drinker or smoker) were excluded from the analysis. The mean follow-up of the remaining 841 women was 9.0 years, and 439 (52.2%) of them completed the follow-up (i.e. their 2008 BP measurements were available); 120 (14.3%) were missing because they had dropped out, and 282 (33.5%) had died (Table 1).

Table 2 shows some of the women's characteristics. Alcohol consumption was generally light: almost all (94%) of the drinkers drank less than on drink/day (data not shown). The frequencies of drinking ($p = 0.894$), smoking ($p = 0.499$) and obesity ($p = 0.793$) were homogeneous across the genotype groups. The women with the $\varepsilon 3/3$ genotype tended to be treated more regularly ($p = 0.087$).

Table 3 shows the MLREPM model estimates of the intercepts and slopes describing the age-related trends of systolic and diastolic BP in the three ApoE genotype groups. In the $\varepsilon 3/3$ group, the expected systolic BP at the age of 60 years was 130.9 mmHg and it increased significantly by a mean of 0.61 mmHg with each year of age, whereas diastolic BP was expected to be about 86 mmHg at the age of 60 years and decreased by an average of 0.28 mmHg at each year of age. There was no substantial difference in BP trends between the genotype groups.

We then evaluated the effect of alcohol consumption on the BP variations with age in the three genotype groups. Too few women used HRT throughout the follow-up, and so the measurements made in conjunction with HRT (56, corresponding to 2.1% of the total) were excluded from the analysis. As smoking status had no appreciable effect on the relationships between ApoE genotype, alcohol consumption and BP, smoking was included in the models as a control variable. Figs. 1–3 show the estimated age-related trends of systolic and diastolic BP for each ApoE genotype group, stratified on the basis of alcohol consumption. Although the difference was not significant, there was a more rapid increase in systolic BP, and a more rapid decrease in diastolic BP among the drinkers in the $\varepsilon 2/3$ than among the non-drinkers (Fig. 1). The younger drinkers in the $\varepsilon 3/3$ group had higher systolic and diastolic BP levels than the non-drinkers: at the age of 60 years, their expected systolic BP was 140.0 mmHg (9.6 mmHg higher than that estimated for the non-drinkers) ($p = 0.032$), and their expected diastolic BP was 92.7 mmHg (7.1 mmHg higher) ($p = 0.006$). These differences disappeared in the older women (Fig. 2). There were no significant difference in the age-related trends of systolic and diastolic BP between the drinkers and non-drinkers in the $\varepsilon 4$ carrier group (Fig. 3).

We next looked for heterogeneities in the obesity strata (BMI< or $\geq 27\ kg/m^2$) in the $\varepsilon 3/3$ and the $\varepsilon 4$ carrier groups; the $\varepsilon 2/3$ group was too small to allow further stratification. The differences in the younger subjects with the $\varepsilon 3/3$ genotype were confined to the non-obese stratum (Fig. 4). The differences in estimated systolic and diastolic BP between the drinkers and non-drinkers at the age of 60 years was respectively 13.7 mmHg ($p = 0.022$) and

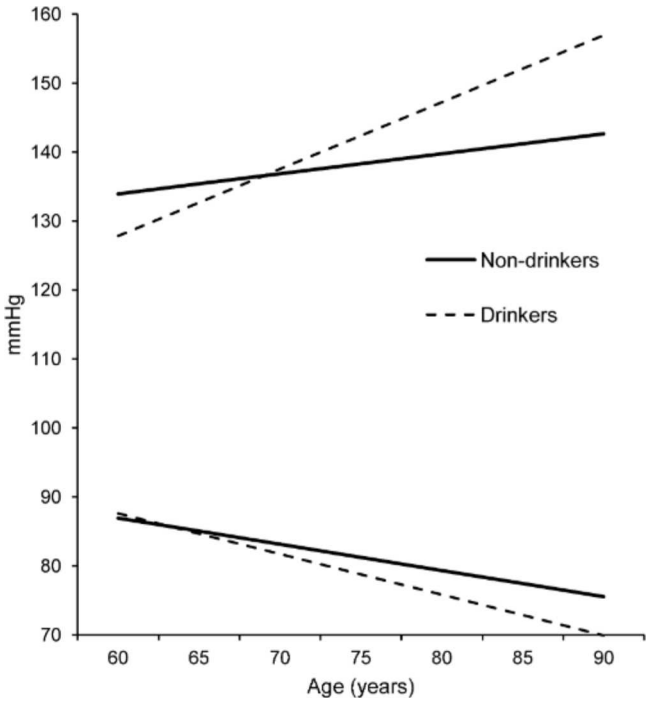

Fig. 1. Estimated age-related trends of systolic and diastolic blood pressure in the $\varepsilon 2/3$ genotype group, stratified on the basis of alcohol drinking habits.

10.7 mmHg ($p = 0.002$). These differences disappeared in the older age groups because systolic BP in the non-drinkers increased more rapidly (0.65 mmHg for each year of age; $p < 0.001$) and because diastolic BP in the drinkers decreased more rapidly (0.81 mmHg), slope significantly different ($p = 0.011$) from that estimated in the non-drinkers.

Among the $\varepsilon 4$ carriers, the BMI strata modified considerably the relationship between alcohol consumption and the age-related

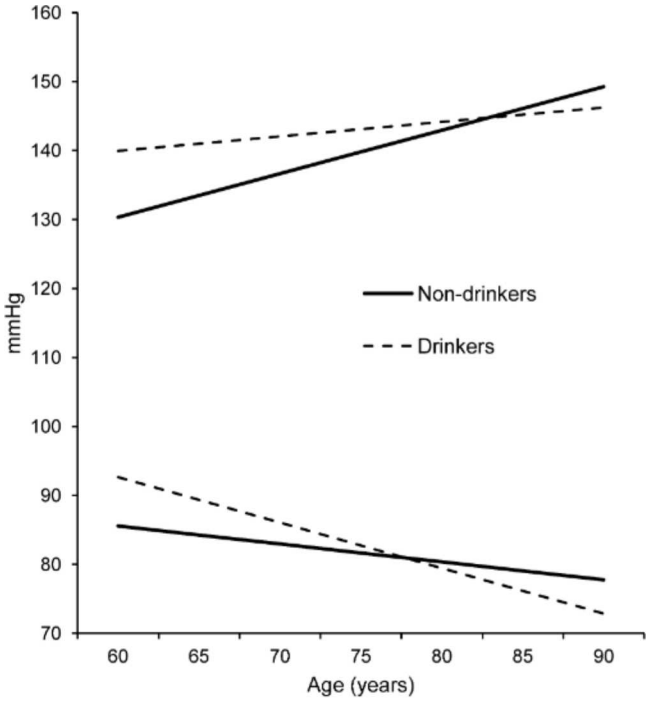

Fig. 2. Estimated age-related trends of systolic and diastolic blood pressure in the $\varepsilon 3/3$ genotype group, stratified on the basis of alcohol drinking habits.

Table 2
Apolipoprotein E genotype distribution, baseline characteristics of the study population, and the use of antihypertensive drugs (AHDs) during follow-up.

	Apolipoprotein E genotype			All subjects
	ε2/3	ε3/3	ε3/4,ε4/4	
No. (%)	94 (11.2)	549 (65.3)	198 (23.5)	841 (100.0)
Mean age (SD), years	68.7 (6.6)	69.3 (7.1)	69.0 (7.4)	69.2 (7.1)
Mean systolic BP (SD), mmHg	136.2 (22.2)	137.3 (22.6)	135.5 (21.3)	136.8 (22.2)
Mean diastolic BP (SD), mmHg	83.2 (12.6)	82.6 (12.2)	82.3 (12.3)	82.6 (12.3)
Percentage of alcohol drinkers	6.4	7.7	7.1	7.4
Percentage of smokers	12.8	9.5	11.6	10.3
Percentage with BMI ≥ 27 kg/m^2	41.3	38.6	37.1	38.6
Percentage of AHD users				
Non-users	17.0	12.8	14.1	13.6
Irregular users	11.7	18.6	21.2	18.4
Frequent users	36.2	26.4	31.3	28.7
Users	35.1	42.3	33.3	39.4

SD: standard deviation; BP: blood pressure; BMI: body mass index.

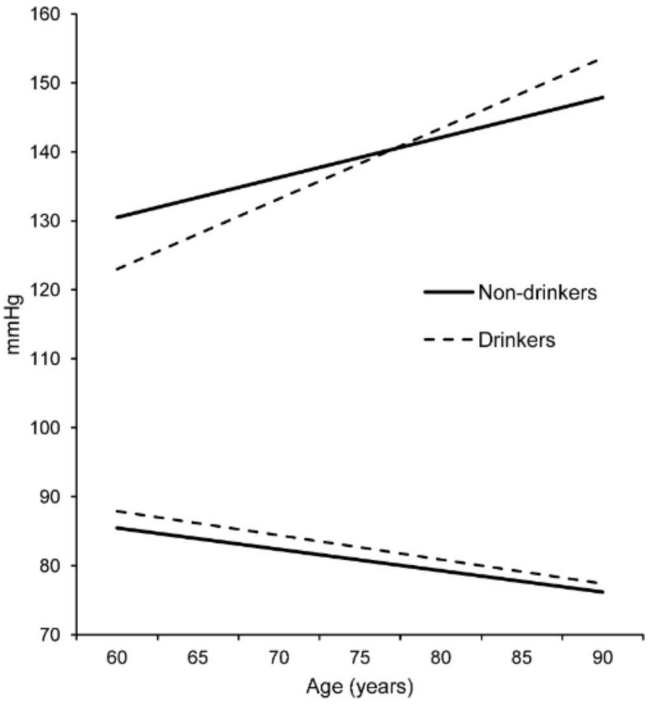

Fig. 3. Estimated age-related trends of systolic and diastolic blood pressure in the ε4 carriers (ε3/4, ε4/4) stratified on the basis of alcohol drinking habits.

trends of BP levels (Fig. 5). In the non-obese group, estimated systolic BP at the age of 60 years in the drinkers was 18.0 mmHg lower than that estimated in the non-drinkers ($p = 0.092$), but increased very quickly: the estimated increase of 1.90 mmHg per year of age ($p = 0.022$) was higher than that of the non-drinkers ($p = 0.091$), leading to expected higher systolic BP in the older age groups. Diastolic BP levels in the drinkers increased with age, leading to expected levels that were 16.9 mmHg higher than those in the non drinkers at 85 years of age ($p = 0.031$).

No significant heterogeneity in BP trends was observed in the obese stratum of the two genotype groups.

4. Discussion

Our findings show the interrelationships between ApoE polymorphism, alcohol consumption and obesity affecting the age-related longitudinal trends of BP levels in postmenopausal women. Alcohol consumption was associated with high levels of systolic and diastolic BP in the younger non-obese women with the ε3/3 genotype and in the older ε4 carriers.

We followed a large number of women for a long time, and the prospective nature of the study represents its main strength as it is the appropriate means of investigating gene–environmental interactions in complex human diseases [20]. We constructed a complex statistical model in order to evaluate the subject-specific BP trajectories accounting for informative (non-ignorable) "missingness". Nevertheless, the analysis was limited by intrinsic imbalances related to the genotype frequencies and a lack of statistical power due to the small size of the drinkers' group. Power is the probability that a statistical test will correctly reject a false null hypothesis. Then, a low statistical power context is of concern when interpreting non-significant results and would not compromise the statistical validity or the meaningfulness of our positive findings. All of the data used in this analysis were updated during the follow-up except for alcohol consumption, for which only baseline data were available; the related estimates may therefore be subject to dilution bias which, however, is known to be toward null and understimates the real association [21].

Although we cannot exclude some degree of under-reporting, practically all of our elderly women were light drinkers. There

Table 3
Age-related linear trends in blood pressure (BP) measurements (mmHg) by apolipoprotein E genotype: estimated[a] values (95% confidence interval).

ApoE	Systolic BP		Diastolic BP	
Genotype	Intercept[b]	Slope[c]	Intercept[b]	Slope[c]
ε2/3	132.8 (126.0, 139.7)	0.38 (−0.13, 0.88)	87.0 (83.1, 91.0)	−0.38 (−0.69, −0.08)
ε3/3	130.9 (127.6, 134.2)	0.61 (0.30, 0.92)	85.9 (84.1, 87.8)	−0.28 (−0.48, −0.07)
ε3/4,ε4/4	129.3 (124.6, 134.0)	0.65 (0.27, 1.03)	85.1 (82.5, 87.8)	−0.28 (−0.52, −0.04)

[a] Using multilevel mixed-effects pattern-mixture models as described in the text.
[b] Expected values at the age of 60 years.
[c] Mean variation in BP levels for each year of age.

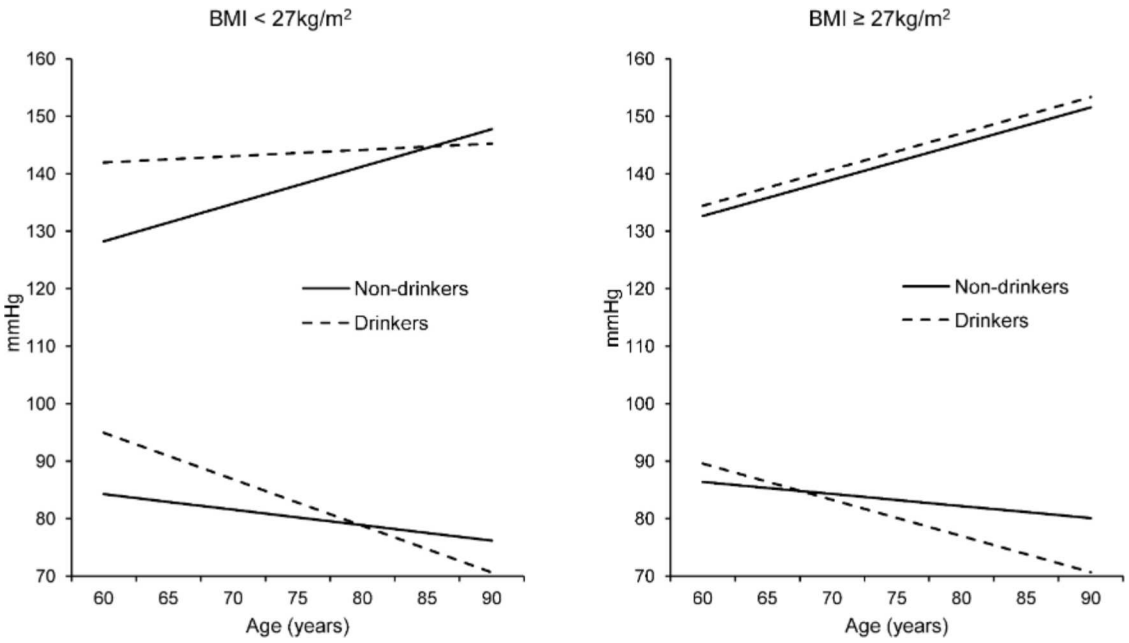

Fig. 4. Estimated age-related trends of systolic and diastolic blood pressure in the ε3/3 genotype group, stratified on the basis of alcohol drinking habits and obesity.

are few data concerning the influence of age on the effect of light drinking, but the elevating effect of alcohol consumption on BP is more prominent in the elderly than in the young [5], and a protective effect of alcohol on hypertension has only been found in young female light drinkers [7]. Experimental data have shown that the hemodynamic effects of alcohol (particularly its hypotensive action) are estrogen-dependent [22], and so the reduced benefit of light–moderate alcohol consumption in postmenopausal women may be related to the decrease in their circulating estrogen levels.

Alcohol intake may affect the BP levels of elderly women by altering the serum concentrations of sex steroids. It has been found that alcohol consumption in postmenopausal women is especially associated with higher serum levels of dehydroepiandrosterone sulphate (DHEAS) and testoterone [23,24], both of which have been shown to be associated with a higher incidence of hypertension and greater longitudinal increase in BP in postmenopausal women [25]. Alcohol consumption may therefore contribute to exacerbating estrogen/androgen imbalances, and changes that favor an increases in androgens and lead to the activation of the renin–angiotensin system (RAS) are among the potential mechanisms responsible for postmenopausal hypertension [26].

In our study, the hypertensive effect of alcohol drinking was confined to non-obese women. After menopause, the main source

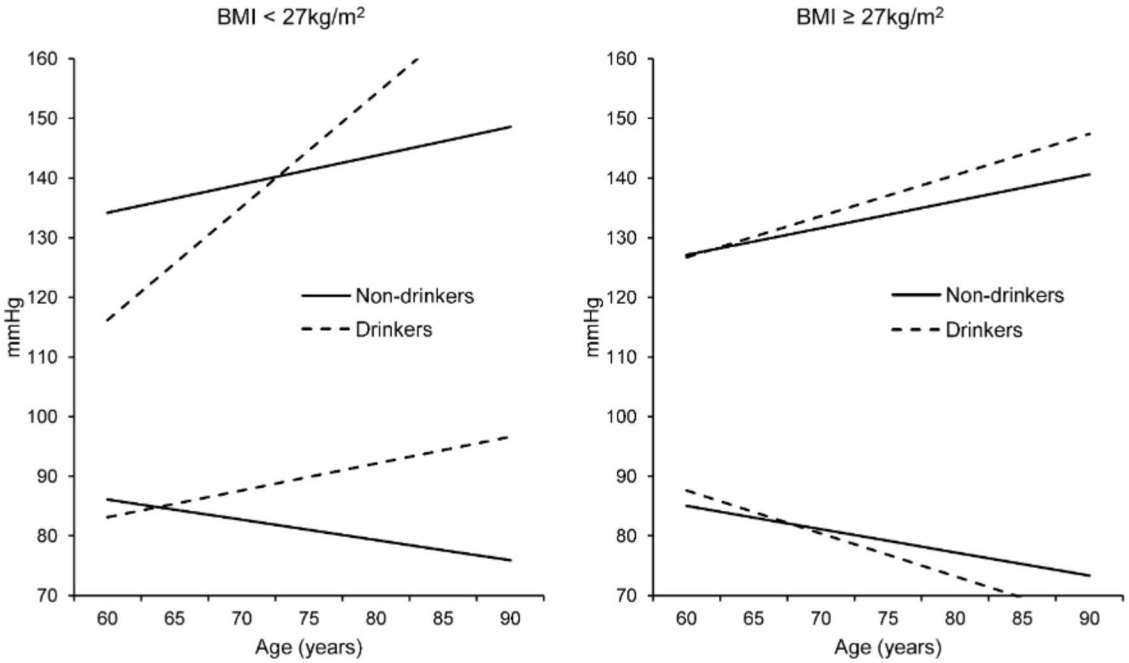

Fig. 5. Estimated age-related trends of systolic and diastolic blood pressure among ε4 carriers (ε3/4, ε4/4), stratified on the basis of alcohol drinking habits and obesity.

of circulating estrogen is the aromatization of androgen precursors to estrogen in adipose tissue [27]. Fatter women have higher levels of aromatase, and so obese postmenopausal women have higher estrogen levels than lean postmenopausal women [28]. It is therefore likely that the estrogen/androgen balance in obese postmenopausal women tends to remain preserved. On the other hand, it is also possible that our BMI cut-off value (<27 kg/m^2) defines a group of women who prevalently drink outside meals, and it has been found that this pattern of drinking has a significant effect on hypertension risk, regardless of the amount of alcohol consumed [29].

Alcohol drinking was associated with high systolic and diastolic BP levels in the younger women with the ε3/3 genotype, and in the older ε4 carriers. The mechanisms underlying these age–genotype interactions are unclear, but it is possible that the typical hormonal changes associated with menopause (particularly the decrease in estrogen levels) is delayed in ε4 carriers. This hypothesis is supported by the observation that age at natural menopause was higher in the ε4 carriers than in the ε3/3 group (p value of the non-parametric equality-of-medians test = 0.023; data not shown). The relationship between ApoE genotype and age at natural menopause, which was older in the ε4 carriers than non-carriers, has also been observed in other populations [30].

Although the limited size of the groups precludes further conclusions, it is interesting to note that the patterns of variation in systolic and diastolic BP levels in our postmenopausal women with the ε2/3 genotype and the obese women in the other two genotype groups were somewhat similar, and suggest that alcohol consumption tends to be associated with a greater increase in pulse pressure. Hypertriglyceridemia is a common trait linking these three groups of women (data not shown) in whom it is possible that alcohol drinking activates non-hormonal, inflammatory and/or oxidative stress pathways, and leads to impaired arterial compliance (see discussion and references in our previous paper [11]).

4.1. Conclusion

In non-obese postmenopausal women, alcohol consumption is associated with systolic and diastolic hypertension, early in those with the ε3/3 ApoE genotype and late in the ε4 carriers. We hypothesize that the effect of alcohol may be mediated by increased androgen levels that change estrogen/androgen ratios and lead to activation of the RAS, and that the observed age–genotype interaction may be due to the influence of the ApoE genotype on age at natural menopause. A better understanding of the mechanisms inducing hypertension in postmenopausal women may lead to better preventive options.

Contributors

Maria Lea Correa Leite: Conception and design of the study, statistical analysis of data, interpretation of data, drafting of the manuscript. Emilio H. Moriguchi: ApoE genotyping, interpretation of data, critical revision of the manuscript. Maria Fernanda Lima-Costa: Design and supervision of the survey, acquisition of subjects and data, conception and design of the study, interpretation of data, critical revision of the manuscript.

All authors have seen and approved the final version of the manuscript.

Competing interest

The authors declare no conflict of interest.

Funding

This study was supported by a grant from the Italian National Research Council (CNR) in the ambit of the Bilateral Agreement of Scientific Cooperation CNR-CNPq (Brazil).

Sponsor's role: None.

Acknowledgements

The authors thank Dr. M. Taufer (CMIC Brazil Clinical Research Laboratory, Porto Alegre, Brazil) for genotyping ApoE.

References

[1] Aronow WS, Fleg JL, Pepine CJ, et al. ACCF/AHA 2011 expert consensus document on hypertension in the elderly; a report of the American College of Cardiology Foundation Task Force on Clinical Expert Consensus Documents. Circulation 2011;123:2434–506.
[2] Klatsky AL, Gunderson E. Alcohol and hypertension: a review. The Journal of the American Society of Hypertensions 2008;2:307–17.
[3] Sesso HD, Cook NR, Buring JE, Manson JE, Gaziano JM. Alcohol consumption and the risk of hypertension in women and men. Hypertension 2008;51:1080–7.
[4] Ohmori S, Kiyohara Y, Kato I, et al. Alcohol intake and future incidence of hypertension in a general Japanese population: the Hisayama study. Alcoholism, Clinical and Experimental Research 2003;26:1010–6.
[5] Wakabayashi I, Araki Y. Influences of gender and age on relationships between alcohol drinking and atherosclerotic risk factors. Alcoholism, Clinical and Experimental Research 2010;34(S1):S54–60.
[6] Thadhani R, Camargo CA, Stampfer MJ, Curhan GC, Willett WC, Rimm EB. Prospective study of moderate alcohol consumption and risk of hypertension in young women. Archives of Internal Medicine 2002;162:569–74.
[7] Snow WM, Murray R, Ekuma O, Tyas SL, Barnes GE. Alcohol use and cardiovascular health outcomes: a comparison across age and gender in the Winnipeg health and drinking survey cohort. Age and Ageing 2009;38:206–12.
[8] Bennet AM, Di Angelantonio E, Ye Z, et al. Association of Apolipoprotein E genotypes with lipid levels and coronary risk. Journal of American Medical Association 2007;298:1300–11.
[9] Mahley RW, Rall SC, Apolipoprotein E. Far more than a lipid transport protein. Annual Review of Genomics and Human Genetics 2000;1:507–37.
[10] Hamet P, Apolipoprotein E. alleles and hypertension: controversy or lack of understanding? Journal of Hypertension 2002;20:1941–2.
[11] Correa Leite ML, Moriguchi EH, Lima-Costa MF. Interactive effects of ApoE polymorphism, alcohol and smoking on age-related trends of blood pressure levels in elderly men: the Bambuì cohort study of ageing (1997–2008). J Hum Hypertens 2013, http://dx.doi.org/10.1038/jhh.2012.70 [e-pub ahead of print].
[12] Kauma H, Savolainen MJ, Rantala AO, et al. Apolipoprotein E, phenotype determines the effect of alcohol on blood pressure in middle-aged men. American Journal of Hypertension 1998;11:1334–43.
[13] Wakabayashi I. Influence of body weight on the relationships of alcohol drinking with blood pressure and serum lipids in women. Preventive Medicine 2009;49:374–9.
[14] Lima-Costa MF, Firmo JOA, Uchoa E. The Bambuì cohort study of ageing: methodology and health profile of participants at baseline. Cadernos de Saude Publica 2011;27(S3):S327–35.
[15] Hixson JE, Vernier DT. Restriction isotyping of human apolipoprotein E by gene amplification and cleavage with Hhal. Journal of Lipid Research 1990;31:545–8.
[16] WHO Collaborating Centre for Drug Statistics Methodology, Guidelines for ATC classification and DDD assignment 2010. Oslo; 2009. p. 282. Available from: http://www.whocc.no/filearchive/publications/2010guidelines.pdf
[17] Hedeker D, Gibbons RD. Application of random-effects pattern-mixture models for missing data in longitudinal studies. Psychological Methods 1997;2:64–78.
[18] Correa Leite ML, Lima-Costa MF, Moriguchi EH. Age-related trends of blood pressure levels by Apolipoprotein E genotype: The Bambuì Cohort Study of Ageing (1997–2008). Hypertens Res 2012, http://dx.doi.org/10.1038/hr.2012.175 [e-pub ahead of print].
[19] StataCorp. Stata: release 11. Statistical Software. College Station, TX: StataCorp LP; 2009.
[20] Manolio TA, Bailey-Wilson JE, Collins FS. Genes, environment and the value of prospective cohort studies. Nature Reviews Genetics 2006;7:812–20.
[21] Clarke R, Shipley M, Lewington S, et al. Underestimation of risk associations due to regression dilution in long-term follow-up of prospective studies. American Journal of Epidemiology 1999;150:341–53.
[22] El-Mas MM, Abdel-Rahman AA. Ovariectomy alters the chronic hemodynamic and sympathetic effects of ethanol in radiotelemetered female rats. Clinical and Experimental Hypertension 2000;22:109–26.
[23] Endogenous Hormones and Breast Cancer Collaborative Group. Circulating sex hormones and breast cancer risk factors in postmenopausal women: reanalysis of 13 studies. British Journal of Cancer 2011;105:709–22.
[24] Rinaldi S, Peeters PH, Bezemer ID, et al. Relationship of alcohol intake and sex steroid concentrations in blood in pre- and post-menopausal women: the

European Prospective Investigation into Cancer and Nutrition. Cancer Causes and Control 2006;17:1033–43.
[25] Wang L, Szklo M, Folsom AR, Cook NR, Gapstur SM, Ouyang P. Endogenous sex hormones, blood pressure change, and risk of hypertension in postmenopausal women; the multi-ethnic study of atherosclerosis. Atherosclerosis 2012;224:228–34.
[26] Yanes LL, Reckelhoff JF. Postmenopausal hypertension. American Journal of Hypertension 2011;24:740–9.
[27] Burger HG. The endocrinology of the menopause. Maturitas 1996;23:129–36.
[28] Freeman EW, Sammel MD, Lin H, Gracia CR. Obesity and reproductive hormone levels in the transition to menopause. Menopause 2010;17:718–26.
[29] Stranges S, Wu T, Dorn JM, et al. Relationship of alcohol drinking pattern to risk of hypertension. A population-based study. Hypertension 2004;44:813–9.
[30] Meng F, Wang Y, Liu J, Zhao J, Liu R, Zhou J. ApoE genotypes are associated with age at natural menopause in Chinese females. Age 2012;34:1023–32.