The effect of age, sex and BMI on the aldosterone-to-renin ratio in essential hypertensive individuals

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Objective: The aldosterone-to-renin ratio (ARR) is widely used as a screening test for primary aldosteronism, but its determinants in patients with essential hypertension are not fully known. The purpose of the present investigation is to identify the impact of age, sex and BMI on renin, aldosterone and the ARR when measured under strict, standardized conditions in hypertensive patients without primary aldosteronism.

Methods: We analysed the data of 423 consecutive hypertensive patients with no concomitant cardiac or renal disorders from two different hospitals (Rotterdam and Maastricht) who had been referred for evaluation of their hypertension. Those who were diagnosed with secondary causes of hypertension, including primary aldosteronism, were excluded from analysis. Patients who used oral contraceptives or had hormonal replacement therapy were excluded as well. Plasma aldosterone concentration (PAC), active plasma renin concentration (APRC) and the ARR were measured under standardized conditions. All measurements were taken in the supine position at 10.00 h in the morning, with one subgroup of patients adhering to a sodium-restricted diet (55 mmol/day) for no less than 3 weeks, and the other subgroup maintaining an *ad libitum* diet. In those who were receiving antihypertensive treatment, all medications were discontinued at least 3 weeks before testing.

Results: In neither group did aldosterone correlate with age. Renin, however, was inversely related to age both during low-salt diet ($P < 0.001$) and during *ad lib* salt intake ($P = 0.05$). This resulted in a significant positive correlation between age and the ARR in both groups. Although on both dietary regimens, PAC and APRC were significantly higher in men when compared with women, the ARR was not significantly different between the two sexes. The age-relationships of renin and the ARR were comparable in men and women on both diets, albeit with greater variability in women. There was an upward trend between BMI and the ARR, which reached statistical significance only in men on low-salt diet. In multivariable regression analysis, age remained the only independent determinant of the ARR.

Conclusion: In our essential hypertensive population, the ARR increased significantly with age but was not affected by sex or BMI.

Keywords: age, aldosterone, aldosterone-to-renin ratio, BMI, renin, sex

Abbreviations: APCC, aldosterone producing cell clusters; APRC, active plasma renin concentration; ARR, aldosterone-to-renin ratio; HR, heart rate; IQR, interquartile range; MUMC, maastricht University Medical Centre; PAC, plasma aldosterone concentration; RAAS, renin-angiotensin-aldosterone system

INTRODUCTION

Although current guidelines recommend measuring the aldosterone-to-renin ratio (ARR) as a screening test for primary aldosteronism [1], sensitivity and specificity of the ARR have a wide and variable range. This could be due to the influence of a variety of physiologic and pharmacologic factors on plasma levels of renin and/or aldosterone. For instance, fluctuations in the renin–angiotensin–aldosterone system (RAAS) are associated with age, with renin and aldosterone being the highest in new-borns and decreasing in a temporal fashion thereafter [2]. In addition, some studies have shown that women have higher ARR values than men, which is more prominent during the luteal phase of the menstrual cycle [3,4]. The effect that BMI has on the ARR has been scarcely investigated, even though a positive correlation has been found between BMI and aldosterone [5]. Finally, several other confounding factors such as medication, posture, race and circadian rhythm have frequently been found to have a significant influence on the ARR [6,7]. For a screening test to be effective, it is essential to know how certain confounders affect the test results in a population without the disease under scrutiny. Unfortunately, however, many, if not all, of the available studies have been conducted with suboptimal standardization of test conditions, which leaves room for the proposition that at least part of the observed variability is methodological rather than biological in origin. The aim

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of the present investigation, therefore, is to assess the effect of age, sex and BMI on renin, aldosterone and the ratio thereof when measured under strictly standardised conditions.

PATIENTS AND METHODS

Study population

We analysed the data from consecutive patients who had been referred for evaluation of their hypertension to either a community-dwelling hospital in Rotterdam or the Maastricht University Medical Centre (MUMC), which both have a secondary and a tertiary referral function. Clinical evaluation included an assessment of left ventricular mass and function, renal haemodynamics, urinary albumin excretion and sediment, and measurements of renin, aldosterone and catecholamines. Patients suspected of having renal artery stenosis were subjected to angiography. We applied a isotonic saline infusion test and, if necessary, computed tomography to rule out primary aldosteronism. Patients with secondary causes of hypertension, including primary aldosteronism, were excluded from the analysis, as were those with concomitant renal or cardiac disease. The usage of oral contraceptives and/or hormonal replacement therapy was not permitted either. Eventually, 423 patients with essential hypertension qualified for inclusion in the present study: 242 from the Rotterdam hospital and 181 from MUMC.

Only patients in whom antihypertensives, if any, could be safely withheld for a period of 3 weeks were included in the study. Informed consent was obtained from all participants and the study was approved by the medical ethical committee of both hospitals.

Procedures

Participants from the Rotterdam hospital were placed on a diet containing 55 mmol of sodium per day for 3 weeks, while participants from the MUMC adhered to their usual ad libitum diet. Sodium intake was verified by measurements of sodium output in three consecutive 24-h urine collections, obtained immediately prior to the test day. The average of the three collections was used for analysis.

All investigations took place in a metabolic ward with constant room temperature. On the day of testing, after an overnight fast and complete bed rest for 10 h, measurements of plasma aldosterone concentration (PAC) and active plasma renin concentration (APRC) were obtained at 1000 h through an indwelling needle into the right antecubital vein. Blood was collected in chilled tubes and spun immediately under cooled conditions and the plasma stored at -80° C until assay.

Throughout the entire period, blood pressure was measured at 5-min intervals with an automatic, oscillometric device (Dinamap, Tampa, Florida, USA), while patients remained in a supine position until the end of the study.

APRC was measured by a two-site direct immunoradiometric assay [8] using a commercial kit (RENIN III Generation; Cisbio Bioassays, Codolet, France). In our hands, its characteristics are sensitivity 2.5 mIU/l, intra-assay variability 4.6% and inter-assay variability 7.6%. We measured plasma aldosterone concentration by solid-phase radioimmunoassay [9] using antibody-coated tubes (DIAsource ImmunoAssays, Louvain-la-Neuve, Belgium). This assay in our laboratory has a sensitivity of 20 pmol/l, an intra-assay variability of 4.3%, and an inter-assay variability of 6.7%.

All hormone concentrations were measured in the respective noncommercial hospital labs. The same two technicians performed all measurements on the two locations, using the same assays throughout the study.

Statistical methods

We used IBM SPSS Statistics 27 (IBM Corporation, Armonk, NY, USA) for the statistical calculations. Because most data did not show a normal distribution, we log-transformed these before analysis, which led to normal distributions in all cases. We applied Pearson correlation analysis to assess the correlation of the ARR with age and BMI. We applied the Kruskal–Wallis statistic to test for differences between men and women. We used multivariable and hierarchical regression analyses to assess the individual contribution of each confounding factor on the ARR and its components and the interactions between such factors. One-way analysis of variance (ANOVA) was used to compare differences in the ARR and its components among BMI categories.

Data are expressed as median and interquartile ranges (IQR). A P value of less than 0.05 was considered statistically significant.

RESULTS

Demographic data of the participants are summarized in Table 1. Except for sodium output, which was higher during *ad lib* salt intake, the data were comparable for the two dietary regimens.

When the results from men and women were combined, PAC did not correlate with age on either of the two dietary regimens. By contrast, APRC showed a significant, inverse correlation with age in the low-salt population ($F = 12.11$; $P < 0.001$) and to a lesser extent also in the patients on *ad lib* salt ($F = 3.75$; $P = 0.05$). This resulted in a significant positive correlation of the ARR with age, both the low-salt ($F = 14.33$; $P < 0.0005$) and in the *ad lib* salt population ($F = 6.24$; $P < 0.02$). During neither of the two dietary regimens did PAC or APRC correlate with BMI. The ARR correlated with BMI in low-salt patients ($F = 8.29$; $P < 0.005$) but not in those on *ad lib* salt.

As summarized in Table 2, under both dietary regimens, PAC and APRC were significantly lower in women than in men, while the ARR did not differ between the sexes. Figure 1 depicts for the two dietary regimens how median values of PAC, APRC and the ARR changed as a function of 5-year categories of increasing age in men and women separately. When broken down by sex, aldosterone levels were not related to age in any of the groups. The age-related decline of APRC, on the contrary, was statistically significant in all four patient groups. The relationship of ARR with age appeared to be more complex. Across the whole age range and on both dietary regimens, the ARR in men rose significantly with age, but posthoc analysis revealed that this rise did not start until the age of approximately 45 years. In women, the ARR exhibited a more

TABLE 1. Baseline characteristics of the patients.

Variable	Low-salt group			*Ad lib*-salt group		
	Men	Women	All	Men	Women	All
N	157	85	242	91	90	181
Age (years)	48 (34–55)	47 (35–54)	47 (34–55)	53 (48–60)	46 (35–54)	51 (41–58)
BMI (kg/m^2)	26 (24–27)	25 (22–27)	26 (23–27)	27 (25–29)	26 (22–29)	26 (24–29)
Plasma sodium (mmol/l)	141 (140–143)	140 (138–142)	141 (139–143)	140 (138–141)	139 (139–142)	139 (138–141)
Plasma potassium (mmol/l)	4.2 (4.0–4.5)	4.1 (4.0–4.2)	4.2 (4.0–4.4)	4.2 (3.9–4.6)	4.1 (3.9–4.3)	4.2 (3.9–4.5)
Plasma creatinine (μmol/l)	94 (85–106)	77 (70–86)	88 (77–99)	94 (83–104)	74 (64–81)	83 (73–98)
Urinary sodium (mmol/day)	54 (34–65)	57 (39–65)	54 (34–65)	164 (121–168)	188 (142–212)	164 (137–184)
Urinary creatinine (mmol/day)	15.1 (13.7–17.1)	10.7 (9.5–12.5)	13.7 (11.0–16.0)	14.5 (12.2 -17.0)	10.3 (9.0 -12.2)	12.2 (10.1–15.1)
SBP (mmHg)	146 (134–160)	150 (128–170)	148 (133–163)	170 (144–192)	160 (148–180)	162 (146–182)
DBP (mmHg)	90 (77–98)	90 (80–102)	90 (80–100)	100 (92–110)	100 (92–110)	100 (92–110)
HR (beats per minute)	72 (64–79)	74 (67–81)	72 (65–80)	68 (64–73)	71 (64–73)	68 (64–73)

Data are expressed as medians with interquartile ranges. HR, heart rate.

variable pattern so that age-related increases just failed to reach statistical significance when the whole age range was considered. However, also in women, the ARR was significantly higher ($P = 0.05$) in women above 45 years as compared to their younger counterparts.

When BMI was divided into three categories with a cut-off of 25, 30 and 35 kg/m^2, respectively, the ARR increased significantly from the lowest to the highest category in men on low salt diet only ($P < 0.05$) but not in the others (Fig. 2).

In multivariable and hierarchical regression analyses, age appeared to be the only independent determinant of the hormonal changes, explaining 26% of the variations in APRC in the low-salt population and 28% of those in the *ad lib* salt population ($P < 0.001$ for both). Age explained 20 and 24%, respectively, of the ARR in both groups ($P < 0.005$ for both). Neither sex nor BMI made any contribution over and beyond that of age.

DISCUSSION

The principle finding of our study is that age is the most important determinant of the ARR in patients with uncomplicated essential hypertension. This is largely due to a decrease in renin levels. Although there is no doubt that renin falls with advancing age (as again corroborated in the present study), age-related changes in aldosterone production are less well defined and far more variable. For instance, although several studies found that aldosterone levels fall with increasing age [10–15], others did not observe a decline in aldosterone at all [16] or only in a male population [17]. In the present study, we could not find an effect of age on aldosterone either. Except for methodological differences between the various studies, this discrepancy could be explained by so-called age-related aldosteronism. It appears that the normal ageing process is associated with reduced expression of CYP11B2 and, hence, diminished aldosterone production. However, at the same time, an increased, yet variable, expression of aldosterone-producing cell clusters (APCC) will lead to enhanced autonomous secretion of aldosterone, which, at least in part, counterbalances the physiological decline in aldosterone release [16,18]. Consequently, plasma concentrations of aldosterone will remain unchanged and may even rise.

Our data further suggest that the rise in ARR does not start before the fourth decade. Thus, it seems as if the functional coupling between renin and aldosterone is well preserved during early life but that a progressive dissociation develops roughly after the age of 40–45 years. This would be consistent with the enhanced expression of APCC, which also is more pronounced above 40 years [16].

Our data show that the relationship between age and the ARR is comparable in the two populations that we studied even though both renin and aldosterone were significantly lower in the patients who had a higher salt consumption. As sodium intake differed by a factor of three between the low-salt and the *ad lib* salt intake, it seems as if sodium has a little influence on the relationship between renin and

TABLE 2. Levels of plasma aldosterone concentration, active plasma renin concentration and aldosterone-to-renin ratio in men and women in both study centres

Variable	Low-salt group			*Ad lib*-salt group		
	Men	Women	All	Men	Women	All
N	157	85	242	91	90	181
PAC (pmol/l)	430 (310–587)	334 (244–522)***	400 (280–557)	350 (210–490)*	265 (140–380)*,†	290 (190–440)
APRC (mIU/l)	26.1 (17.5–36.2)	21.0 (12.9–31.0)†	24.9 (15.7–35.0)	21.3 (12.0–32.9)**	14.3 (10.1–24.5)**,†	18.0 (10.4–28.4)
ARR	15 (11–26)	15 (10–27)	15 (11–27)	18 (9–26)	15 (9–27)	16 (9–27)

Data are expressed as medians with interquartile ranges.
APRC, active plasma renin concentration; ARR, aldosterone-to-renin ratio; PAC, plasma aldosterone concentration.
*$P < 0.001$.
**$P < 0.03$ as compared to the low-salt group.
***$P < 0.005$.
†$P < 0.05$ as compared to men.

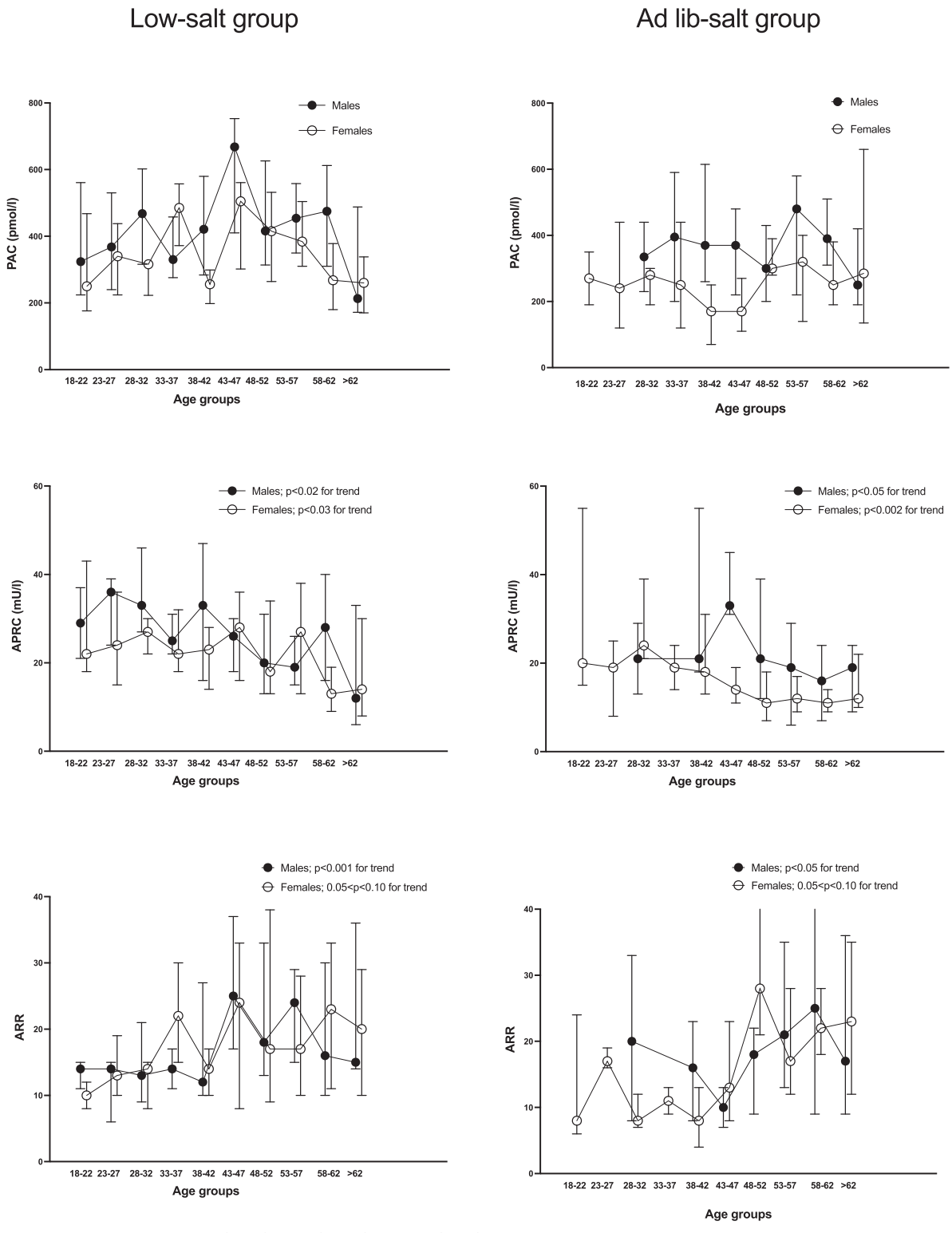

FIGURE 1 Relationship between aldosterone (upper), renin (middle) and ARR (lower) per 5-year age-groups in men and women on two dietary regimens. APRC, active plasma renin concentration; ARR, aldosterone-to-renin ratio; PAC, plasma aldosterone concentration.

aldosterone. Nevertheless, we cannot exclude the possibility that a more extreme difference in dietary sodium would have altered our findings.

Although a rise of the ARR with age was seen in both sexes, our data suggest that in women, the ARR is much more variable across the various age groups. This could explain why some studies found higher ARR in women as compared to men [19,20], while others did not or only in specific subgroups [16,17].

The relationship between BMI and the ARR appears to be inconsistent. Although some investigators found an upward trend of PAC with increasing BMI in hypertensive

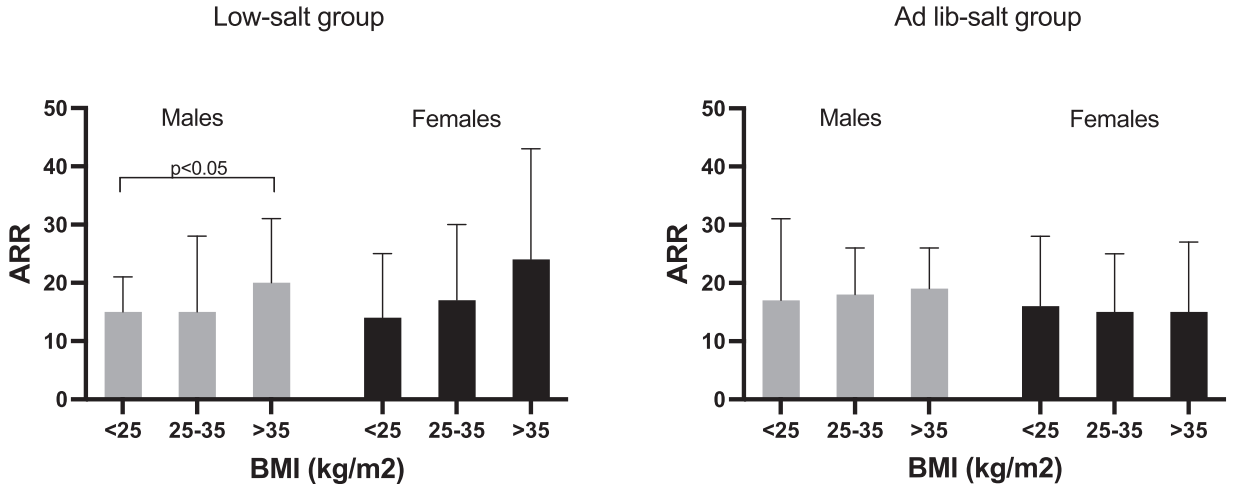

FIGURE 2 The aldosterone-to-renin ratio in three categories of BMI.

patients [5,21], this was not seen in the Framingham Heart Study [22]. In our series, a positive relationship between the two variables was present only in men on low salt intake and we cannot exclude the possibility that this finding arose by chance.

Although most of the studies were performed under well standardised conditions, confounding factors such as sex, age and BMI were often not controlled for. In addition, the usage of some medications such as nondihydropyridine calcium channel blockers and alpha blockers was often permitted [10,11,13,23]. The strength of our study is that all patients were untreated and that the conditions of sampling were rigorously controlled with particular attention to body posture and bed rest prior to the measurements.

The main limitation of the present investigation was the lack of details regarding the female population's menstrual cycle. We expect this to not have a significant influence on the validity of the results as no significant difference in the ARR between the follicular and luteal phases has been reported [3,4]. Another limitation may be that blood samples were only taken in the supine position. We chose to standardize posture to supine, as it has been reported that it causes less variability in ARR measurements [7]. Moreover, our study was not intended to evaluate the validity of the ARR but rather to understand how age, sex and the BMI could affect the relationship between renin and aldosterone in hypertensive patients.

In conclusion, in our essential hypertensive population, the ARR significantly rose with advancing age, particularly after the fourth decade. This rise occurred in both sexes, albeit with greater variability in women. The implication of our finding is that ARR cut-off points may have to be adjusted for age.

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Conflicts of interest

There are no conflicts of interest.

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