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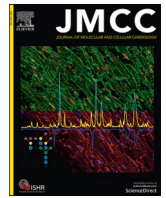
Resting membrane potential is less negative in trabeculae from right atrial appendages of women, but action potential duration does not shorten with age

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ABSTRACT

Aims: The incidence of atrial fibrillation (AF) increases with age. Women have a lower risk. Little is known on the impact of age, sex and clinical variables on action potentials (AP) recorded in right atrial tissue obtained during open heart surgery from patients in sinus rhythm (SR) and in longstanding AF. We here investigated whether age or sex have an impact on the shape of AP recorded in vitro from right atrial tissue.

Methods: We performed multivariable analysis of individual AP data from trabeculae obtained during heart surgery of patients in SR ($n = 320$) or in longstanding AF ($n = 201$). AP were recorded by sharp microelectrodes at 37 °C at 1 Hz. Impact of clinical variables were modeled using a multivariable mixed model regression.

Results: In SR, AP duration at 90% repolarization (APD_{90}) increased with age. Lower ejection fraction and higher body mass index were associated with smaller action potential amplitude (APA) and maximum upstroke velocity (V_{max}). The use of beta-blockers was associated with larger APD_{90}. In tissues from women, resting membrane potential was less negative and APA as well as V_{max} were smaller. Besides shorter APD_{20} in elderly patients, effects of age and sex on atrial AP were lost in AF.

Conclusion: The higher probability to develop AF at advanced age cannot be explained by a shortening in APD_{90}. Less negative RMP and lower upstroke velocity might contribute to lower incidence of AF in women, which may be of clinical relevance.

1. Introduction

Atrial fibrillation (AF) is the most common rhythm disorder and associated with high comorbidity and increased mortality risk [1]. Research in recent years yielded detailed information about epidemiology and comorbidities affecting AF [2]. The incidence of AF increases with age, and women are at lower risk of developing the disease [1,3,4].

Many classical cardiovascular risk factors and comorbidities, e.g. heart failure, myocardial infarction, sleep disordered breathing, are known to affect AF [4]. However, their impact on atrial electrophysiology remains unclear. A more detailed understanding of the underlying pathophysiological mechanisms would be a further step towards improving personalized medicine [2]. Therefore, we set out to investigate retrospectively the impact of clinical variables on action potentials (AP)

recorded in right atrial tissue obtained during open heart surgery from patients in sinus rhythm (SR) and in persistent atrial fibrillation (AF).

2. Methods

2.1. Human tissue samples

The study followed the declaration of Helsinki and all patients gave written informed consent. All investigations were approved by the Medical Faculty Ethics Committee of the Technical University Dresden; approval number EK 114082202. Each patient gave written, informed consent. Clinical parameters were collected from the medical records of patients and analyzed after anonymisation. Part of data were previously used for a preliminary analysis on sex effects on AP [5]. Some data were used for machine learning algorithm. [6]

Data were retrospectively analyzed from 521 patients undergoing cardiac surgery. 320 patients with SR and 201 patients with longstanding persistent AF were included in this analysis. After excision, tissue was immediately placed at room temperature into a non-oxygenated cardioplegic solution (in mM): NaCl 100, taurine 50, glucose 20, KCl 10, $MgSO_4$ 5, MOPS (3-(N-morpholino) propanesulfonic acid) 5, KH_2PO_4 1.2, containing 30 mM of the myosin ATPase inhibitor BDM (2,3-butanedione monoxime) and transferred to the laboratory in <10 min.

2.2. AP recordings

APs were recorded with standard intracellular microelectrodes in atrial trabeculae. Bath solution contained (in mM): NaCl 127, KCl 4.5, $MgCl_2$ 1.5, $CaCl_2$ 1.8, glucose 10, $NaHCO_3$ 22, NaH_2PO_4 0.42, equilibrated with O_2-CO_2 [95:5] at 36.5 ± 0.5 °C, pH 7.4. Preparations were regularly stimulated for at least 1 h before data acquisition with a custom-made computer program (University of Szeged, Hungary) that
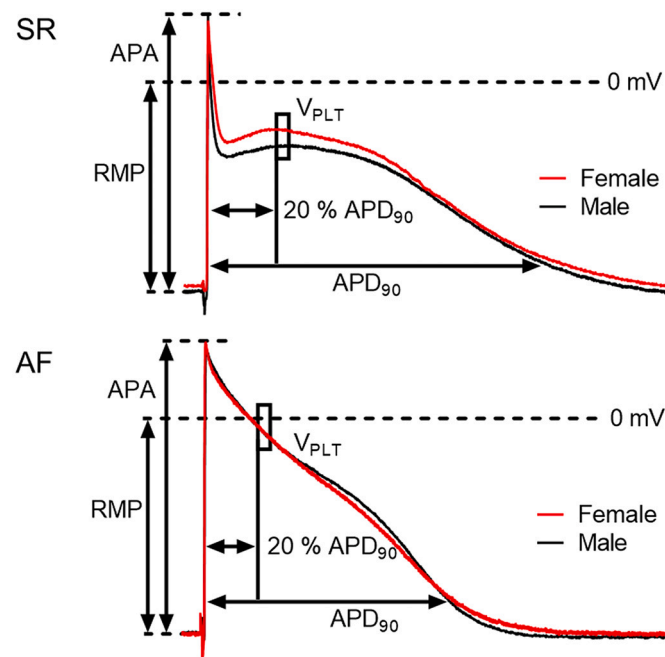
also generated electrical stimuli. The following AP parameters were analyzed (see Fig. 1): action potential amplitude (APA), resting membrane potential (RMP), AP duration at 20, 50, and 90% of repolarization (APD_{20}, APD_{50}, APD_{90}, respectively), plateau potential (V_{PLT}) defined as the mean absolute membrane potential in a 5-ms window starting from 20% of APD_{90}, and maximum upstroke velocity (V_{max}). APs were analyzed offline by using the LabChart® software (ADInstruments, Spechbach, Germany).

2.3. Statistics

Raw data for AP characteristics for male and female in SR and AF are plotted in Supplement Figures S1 and S2. Patient characteristics are presented as mean $+/-$ standard deviation and as absolute and relative frequencies. Multivariable analysis using mixed models are state of the art to measure the impact of clinical characteristics on outcome in epidemiological studies. Mixed models have remained uncommon in the analysis of in vitro studies, since sample size is often too small to allow application of multivariate analysis. Mixed models were fit in order to find associations between patient and/or measurement characteristics and measurement (ADP_{20}, ADP_{50}, ADP_{90}, VPLT, RMP, APA, V_{max}). Multivariable mixed model regressions were performed, each time including age and sex as fixed effects and a possible third variable included in the model as a third fixed effect. Between-patient variability was included as a random effect. The beta coefficients obtained in the mixed model regressions represent change of measurement value.

For the different types of measurements, predicted values are given in Table 2. These predictions are based on the mixed-model regression [7,8] with random within and between patient effects and fixed effects according to the following statistical model measurement ~ Sex + AF + (Interaction of Sex and AF). Standard errors, as estimated using mixed-model regression are given, and these should be seen as confidence bands. The *p*-values in Table 2 were calculated using Wald test, the coefficients were estimated by the mixed model and their standard errors. Assuming normally distributed Wald statistics with mean zero and standard deviation one in the null hypothesis, the Wald tests were implemented using the standard normal or z-distribution [9].(See Fig S1 and S2).

3. Results

We analyzed data from 521 patients undergoing open heart surgery. 320 patients with SR and 201 patients with longstanding persistent AF were included in this retrospective analysis.

3.1. Clinical characteristics of patients in SR

170 (53.1%) patients underwent isolated coronary bypass grafting (CABG), 94 (29.4%) received isolated valvular surgery and 56 (17.5%) had combined CABG and valve procedures. The mean patient age was 67.2 ± 10.2 years, 72.2% of patients were men. Mean body mass index (BMI) was 27.5 kg/m^2. 38.6% of patients had diabetes mellitus. Mean left ventricular end diastolic diameter (LVEDD) was 50.7 mm and left ventricular ejection fraction (LVEF) was 53.5%. Left ventricular end diastolic pressure was 18.2 mmHg and left atrial size was 43 mm. Medication included Beta-blocker treatment in 83.9% of patients. The majority of patients were treated with ACE-inhibitors (60.8%) or AT$_1$-blockers (24.4%). 49.1% of patients received diuretics, 71% were treated with statins. Details regarding clinical characteristics and medication of the patients are given in Table 1.

3.2. Clinical characteristics of patients in AF

Isolated valve surgery was performed in 133 (66.2%) patients, isolated CABG in 19 (9.4%) patients and 49 (24.4%) patients were treated with combined CABG and valve surgery. Mean age in the AF group was

Fig. 1. Superimposed action potentials from a male and a female patient in sinus rhythm (top, SR) and in atrial fibrillation (bottom, AF) to illustrate analysis of action potential parameters. APA action potential amplitude (mV); RMP resting membrane potential (mV), APD_{20}, APD_{50}, and APD_{90}, action potential duration at 20, 50, and 90% of repolarization (in ms), and the "plateau potential" defined as the mean absolute membrane potential (in mV) in the time window following 5 ms after 20% of APD_{90} (V_{PLT})."

Table 1

Patient characteristics.

	SR ($n = 320$)	AF ($n = 201$)
Patient characteristics		
Age (years)	67.2 ± 10.1	72.5 ± 7.3
Sex, male n (%)	231 (72.2)	126 (62.7)
Body mass index kg/m^2	27.5 ± 4.1	28.0 ± 4.3
Hypertension n (%)	297 (94.6)	192 (97.0)
Diabetes mellitus n (%)	123 (38.6)	79 (39.9)
Hyperlipidemia n (%)	237 (75.5)	130 (70.3)
Coronary artery disease n (%)	244 (76.3)	94 (46.8)
Valvular disease n (%)	154 (48.1)	175 (87.1)
Left ventricular ejection fraction (%)	53.5 ± 12.6	52.1 ± 11.9
Left ventricular enddiastolic diameter (mm)	50.7 ± 7.8	51.9 ± 7.7
Left ventricular enddiastolic pressure (mm Hg)	18.2 ± 8.0	16.4 ± 6.3
Left atrial diameter (mm)	42.8 ± 5.9	51.2 ± 8.2
Left ventricular hypertrophy n (%)	165 (53.7)	119 (63.6)
Medication		
Beta-blockers n (%)	265 (83.9)	173 (86.5)
Digitalis n (%)	9 (2.9)	61 (30.3)
Ca^{2+}-channel-blockers n (%)	63 (19.9)	43 (21.7)
ACE-inhibitors n (%)	192 (60.8)	130 (66.0)
AT$_1$-blockers n (%)	77 (24.4)	54 (27.3)
Diuretics n (%)	155 (49.1)	152 (76.8)
Statins n (%)	233 (71.0)	110 (55.8)
Nitrates n (%)	51 (16.1)	21 (10.6)

Data are in mean ± standard deviation or numbers (%).

72.5 ± 7.3 years, 62.7% were male. Mean BMI was 28.0 kg/m^2 in the AF group and 39.9% of patients had diabetes mellitus. Mean left ventricular ejection fraction was with 52.1% slightly lower than in SR (Table 1) and mean left ventricular end diastolic diameter was 51.9 mm. Mean left-atrial diameter was 51.2 mm in the AF group and significantly larger than in the SR group (Table 1). Left ventricular end diastolic pressure was 16.4 mmHg in the AF group. 86.5% of patients in AF group were treated with beta-blockers, while 30.3% of patients received digitalis treatment. ACE inhibitor treatment was present in 66% of patients, while 27.3% received AT$_1$-blocker treatment. 76.8% of AF patients were treated with diuretics, while 55.8% had statins in their medication. Detailed information on clinical characteristics and medication are given in Table 1.

3.3. AP parameters for RAA preparations from patients in SR and AF

In a first step we plotted AP parameters recorded in preparations from patients in SR and AF (Supplement Figs. 1 and 2). Data for APD$_{20}$ and APD$_{90}$ showed large data scattering in SR but not in AF. We did not exclude such "outliers".

3.4. Effects of age and sex on AP shape

In order to visualize potential impact of age and sex on AP shape we simply plotted AP parameters against age for data obtained from female and male patients in SR and AF (Figs. 2 and 3). In AF regression lines for preparations from male and female patients were almost superimposable, while in SR there was a hint for less negative RMP and lower APA and V$_{max}$ in preparation from women. In SR age was associated with longer APD$_{90}$ (both female and male) but not in AF.

3.5. Multivariable analysis of patients with SR

To dissect independent impact of different clinical variables on AP shape we performed a multivariable analysis, based on 370 samples from 320 patients in SR (Fig. 4). To validate our model we performed a normal probability plot of the residuals. Residuals are the difference between any data point and the model-predicted regression line. In other words, the residual is the error that isn't explained by the

regression line. If the normal probability plot of the residuals is approximately linear one can assume that the error terms are normally distributed and hence application of a linear model is justified. Typical normal probability plots are given for age, sex and BMI in Supplement Fig. 3 and 4 (for other clinical parameters plots not shown). Only for APD$_{50}$ and APD$_{20}$ normality plots (for any clinical variable) are skewed and may have therefore limited statistical power to detect an association. Calculated estimated beta values are given in Fig. 4 and 5 and represent the change in the APD parameter value per unit increase (or decrease) in the respective clinical variable when all the other clinical variables are held constant.

Female sex was associated with significantly less negative RMP, smaller APA and smaller V$_{max}$. We observed a slight but significant increase of APD$_{90}$ with age. Unexpectedly, longer QT$_c$ was not associated with longer APD$_{90}$ but not with longer APD$_{20}$ (and less negative V$_{PLT}$). Female sex was not associated with longer APD$_{90}$, but APD$_{20}$. Presence of coronary artery disease, left-ventricular hypertrophy and treatment with beta-blockers was associated with longer APD$_{90}$. Lower EF and higher BMI were associated with smaller APA and V$_{max}$ while APD was not affected. Detailed results of multivariable analysis are displayed in Supplemental Table S1.

3.6. Multivariable analysis of patients with AF

In the AF cohort, 287 samples from 201 patients were included in a multivariable analysis (Fig. 5). As seen in SR, there was an association of QT$_c$ and APD$_{20}$ as well as QTc and V$_{PLT}$. However, there was no effect of sex on any AP parameter. Increasing age was associated with shorter APD$_{20}$; no effect was detectable on APD$_{90}$. Furthermore, in contrast to SR, BMI and lower EF did not associate with APA or V$_{max}$, but were associated with shorter APD$_{90}$. Treatment with digitalis glycoside (not present in SR at all!) was associated with shorter APD (APD$_{20}$ and APD$_{50}$ but not APD$_{90}$) and smaller APA. No association was seen between treatment with Ca-channel blockers, ACE-inhibitors, AT$_1$-blockers, diuretics, statins and nitrates on any AP parameter (neither in SR nor in AF). Smaller APA associated also with hyperlipidemia. Additionally, V$_{max}$ was smaller in patients with higher LVEDP. Detailed results of multivariable analysis in patients with AF are shown in Supplemental Table S2.

3.7. Interaction between rhythm status and sex

To visualize interaction between rhythm status and sex we performed 3D histograms (Supplement Fig. 5). Numerical data and the respective statistics are given in Table 2. In both women and men APD$_{90}$ was shorter and APD$_{20}$ was longer in AF than in SR. RMP was more negative and APA and upstroke velocity were higher in both preparations from female and male patients in AF compared to SR.

4. Discussion

4.1. Principal findings

In our large data set we investigated the impact of clinical variables on human atrial AP by multivariable analyses. We found only moderate effects of age on APD$_{90}$, which we assume cannot explain the steep increase in AF prevalence with age. However, there were clear sex-related differences that could be relevant for the lower incidence of AF in women.

4.2. Clinical context: age

The higher likelihood of AF observed in the elderly suggests distinct electrophysiological changes that facilitate the occurrence of AF. Effects of age on atrial electrophysiology are known for long time. Under in vivo conditions measured effective refractory period (ERP) in right atrium is
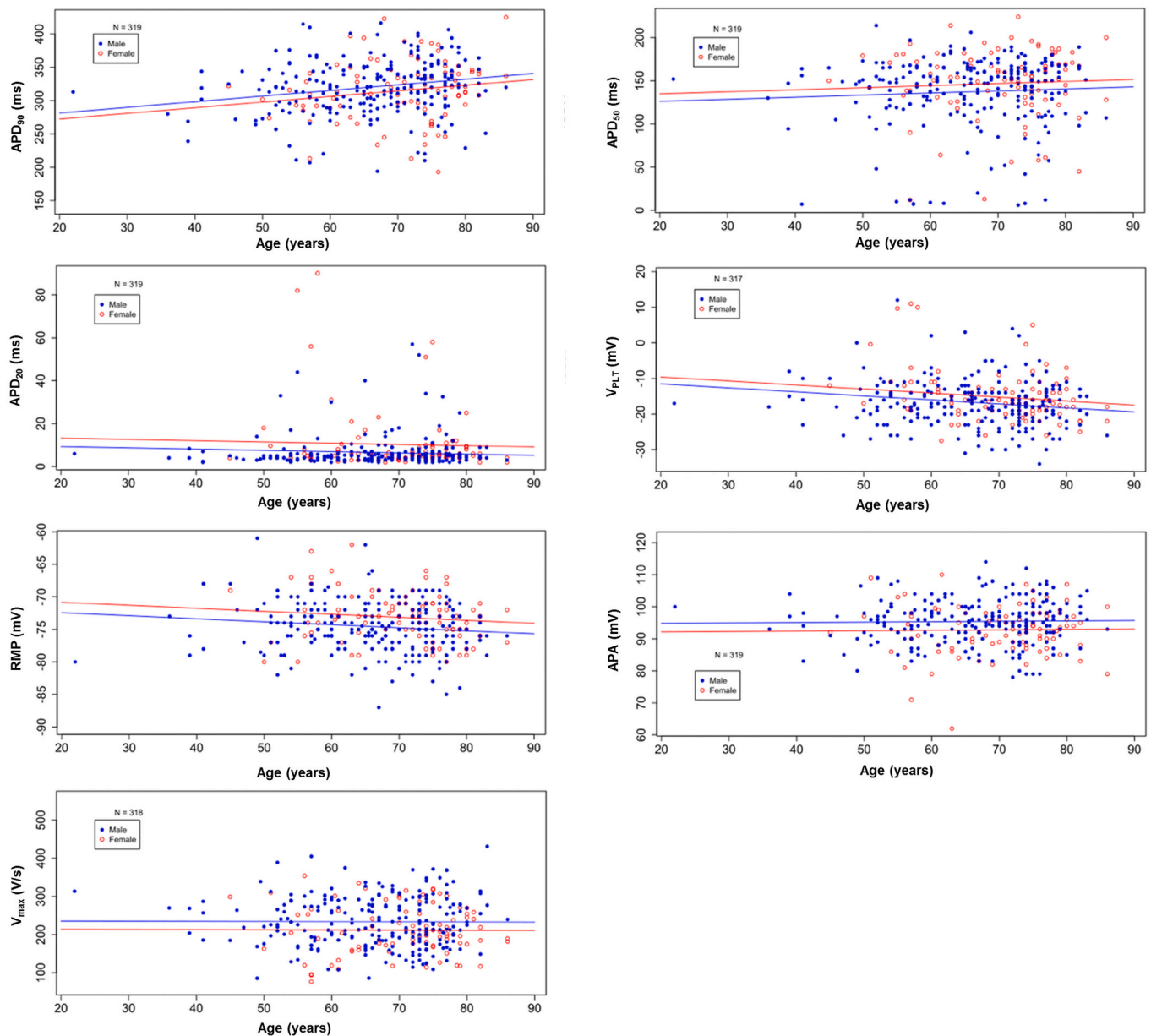
Fig. 2. Individual data points for action potential duration at 20, 50, and 90% of repolarization (APD_{20}, APD_{50}, and APD_{90}), plateau voltage (V_{PLT}), resting membrane potential (RMP), action potential amplitude (APA) and maximum upstroke velocity (V_{max}) right atrial appendages from male and female patients in SR plotted against age (n = 320, fit from multivariate analysis).

shorter in children than in adults [10]. In line with this finding in vitro APD_{90}, measured by sharp microelectrodes in human preparation from right atrial appendages from children is shorter compared to tissues from adults [11]. Age-dependent effects on atrial electrophysiology in adults are available from in vivo ERP studies only. Michelucci et al. reported in a rather small study a longer ERP (~30 ms) in older vs. young adults [12]. Three other independent groups confirmed the slightly longer right atrial ERP in older adults [13–15]. However it must be noted that one study did not report any effect of age on right atrial ERP [16]. Reason for that discrepancy remains unclear. ERP is frequently taken as a surrogate parameter for APD_{90} but there is no one to one relationship. In addition, mentioned above studies used rather simple statistical methods (comparing rather arbitrary age groups, application of simple linear regression). The multivariable approach we used in this study should allow direct age-related effects to be distinguished from effects of an age-associated increase in comorbidities or other variables. In this context, it seems worth to mention that our

results in human atria fit almost perfectly to earlier results obtained in healthy dogs where APD_{90} in old dogs was also slightly longer (20 ms) [17]. Mechanism of age-dependent increase in APD_{90} observed in this study remains to be elucidated. In both human (this study) and dog right atrium older age does not only associate with longer APD_{90} but also with lower V_{PLT} (8 mV in dogs, method for assessing plateau potential is not clearly stated, APD_{30} quite similar). Since at least in human atrium less negative V_{PLT} leads to stronger contribution of I_{Kr} to repolarization [18], it remains attractive to suspect a link between V_{PLT} and APD_{90} in age dependent prolongation of APD_{90}. Lower V_{PLT} could result either from lower $I_{Ca,L}$ and/or larger transient outward potassium currents (I_{to} and I_{Kur}). In fact, at least one study reported lower protein expression of $Ca_{V1.2}$ in tissue from older patients [19]. No such data is available for transient potassium currents. Interestingly in the same study, protein expression of Kir2.1 and Kir2.3 in human atrium was not changed with age [19], fitting nicely to the absence of age-dependent effect on RMP (this study). In stark contrast to an animal model, we did not observe a
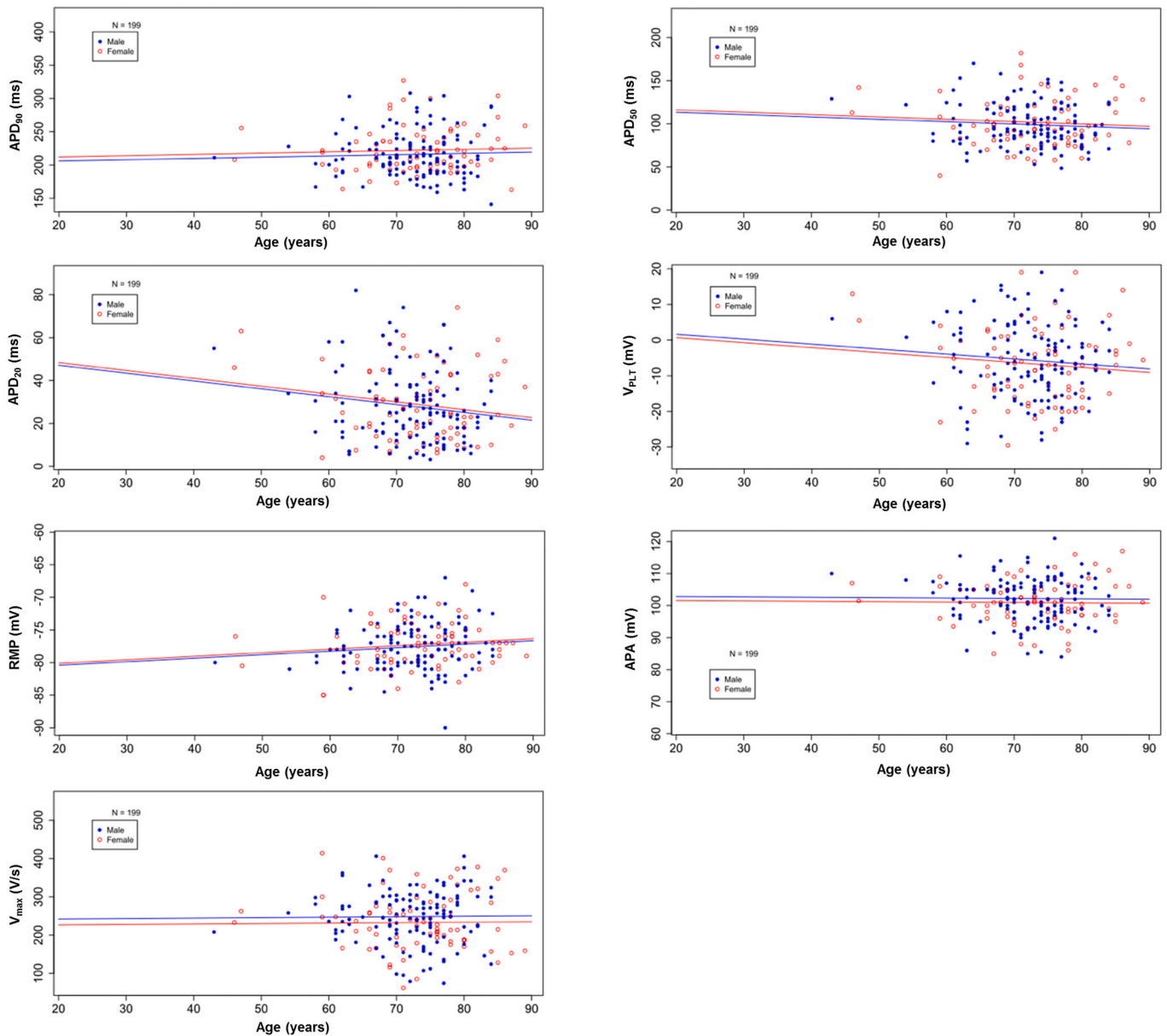
Fig. 3. Individual data points for action potential parameters (same abbreviations as in Fig. 2) in AF (n = 201, fit from multivariate analysis).

decrease in maximum upstroke velocities with age [20]. Thus, structural changes may underlie the well-described conduction slowing atria in elderly humans [21]. From a clinical perspective, we would not expect a relevant contribution of age-dependent prolongation in APD_{90} to AF. In our data set APD_{90} increases rather monotonically over age, while AF incidence sharply increases over 70 years [4]. More importantly, APD_{90} prolongation would rather act as a protective mechanism. Anyhow, our data and the mentioned above studies on ERP show that the age-dependent shortening in APD_{90} and/or ERP found in rodents [22,23] does not translate into human biology.

4.3. Clinical context: sex (atrial APD)

There is no ECG parameter available that allows to draw conclusions as to atrial APD. No data on sex effects on APD_{90} measured in vitro are published so far. Absence of sex effects on atrial APD_{90} (this study) is in line with two in vivo studies that report no difference in atrial ERP between man and women [15,16]. These findings are somewhat surprising, since one would expect that atrial APD_{90} follows ventricular APD_{90}.

With the onset of puberty women develop a longer QT interval (reflecting ventricular APD_{90}) due to hormonal influence on ion channel expression [24]. If this holds true, longer QT in women should be associated with longer atrial APD_{90}. However, we did not find such an association. Unexpectedly, APD_{20} associated with QT (both in men and women) and was larger in women suggesting fundamental differences in regulation of APD between human ventricle and atrium. Relevance of our findings for refractoriness is hard to judge, since in human atrium APD_{75} but not APD_{90} coincides with ERP [25]. Here we found a trend for longer APD_{90} in tissue from women vs. men, while the differences in APD_{20} and APD_{50} reach the level of significance. One would expect data for APD_{75} between APD_{90} and APD50. Therefore, it remains justified to speculate whether atrial ERP is longer in tissue from women vs. men.

4.4. Clinical context: sex (RMP)

Most of the studies investigating sex-related differences in cardiac electrophysiology focus on effects of calcium currents and of main repolarizing potassium currents on APD in ventricles (see above) [3].
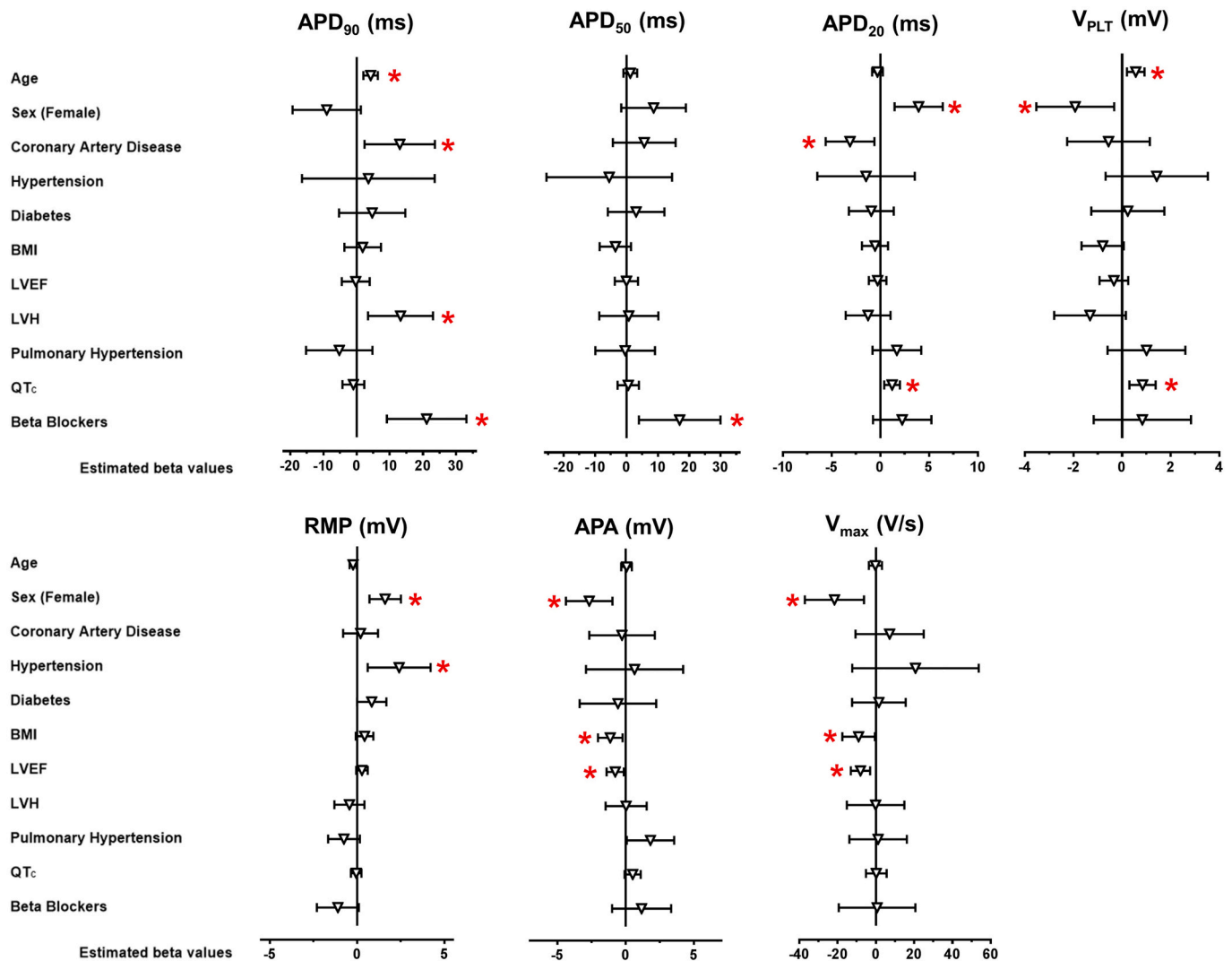
Fig. 4. Effects of clinical variables on action potential parameters in SR.
Forest plot of estimated beta effect values and associated 95% confidence intervals of clinical variables and drug treatment on action potential duration at 20, 50, and 90% of repolarization (APD_{90}, APD_{50} and APD_{20}), plateau voltage (V_{PLT}), resting membrane potential (RMP), action potential amplitude (APA) and maximum upstroke velocity (V_{max}). n = 370/320 number of trabeculae/number of patients. Multivariable model, described in methods section, was used to calculate p-values. For exact confidence intervals, compare Supplement Table 1. * indicates $p < 0.05$.

Less is known about sex-dependent regulation of RMP. Ventricular cells from female rabbits and guinea-pigs exhibited less I_{K1} than those from male animals, but no data on RMP and V_{max} were reported. [26,27] In human ventricular myocytes there is no evidence for smaller I_{K1} in cells from women. Furthermore, there was only a trend to less negative RMP in cells from women; V_{max} was not different [28]. Here we found a 2 mV difference in atrial RMP between males and females, which associated with a 10% higher V_{max}. V_{max} is proportional to the square of conduction velocity in heart tissue [29]. Therefore, our results suggest slower conduction in atrial tissue from women vs. men. The lower V_{max} can at least partially be explained by the difference in RMP [30]. Therefore, our results suggest that less inward rectifier currents in women may be the most relevant sex-dependent difference in human atrium. A less negative RMP should not only reduce upstroke velocity at the low pacing rate used here (1 Hz), but also delay recovery of sodium currents form inactivation and thereby prolong refractoriness [31]. Pharmacological block of inward rectifier currents is effective against atrial fibrillation in well-characterized animal models [32]. Effects of class I antiarrhythmics on sodium channels are larger at less negative RMP [33]. One might speculate that lower RMP in women's atria does not only reduce AF but

may facilitate drug effects on sodium channels. In fact, two randomized studies showed higher efficacy of vernakalant (mainly acting via sodium channels [34,35]) to convert AF in women [36,37]. Recent work suggests clinically used antiarrhythmics may differ with respect to their voltage-dependence of sodium channel block [38]. It remains to be proven, whether compounds with stronger voltage dependence may have larger effects in women.

4.5. Clinical context: diabetes mellitus

38.6% and 39.9% of patients had diabetes mellitus in SR and AF group, respectively. Despite relevant prevalence of diabetes mellitus we could not detect any impact on AP parameters. This finding is at variance to early studies in rat hearts where atrial APD_{90} was longer in streptozotocin-induced diabetes [39]. However, in a clinically more relevant overfeeding model of diabetes in rat, APD was not changed, but inducibility of AF was enhanced. [40]
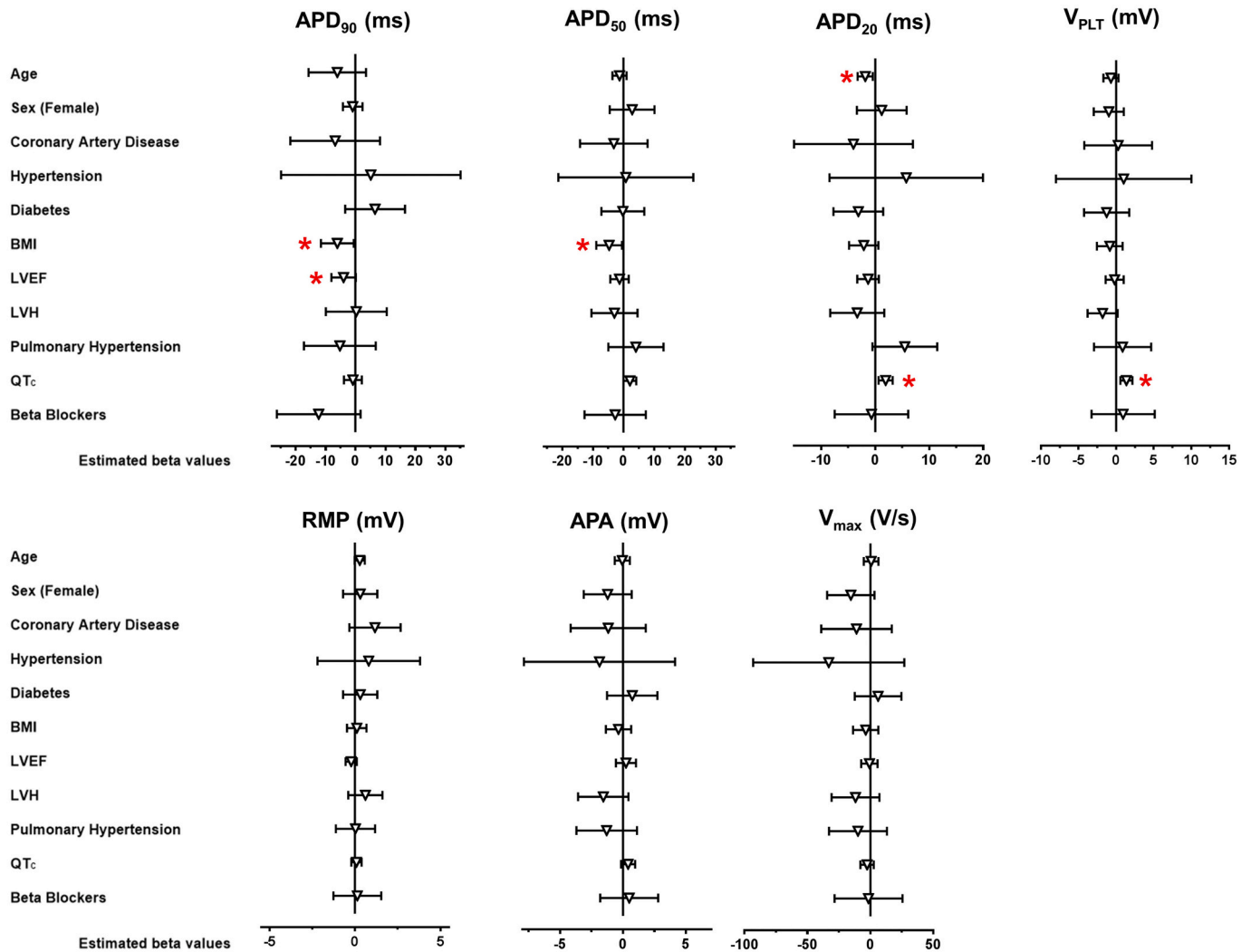
Fig. 5. Effects of clinical variables on action potential parameters in AF.
Forest plot of estimated beta effect values and associated 95% confidence intervals of clinical variables and drug treatment on action potential duration at 20, 50, and 90% of repolarization (APD_{90}, APD_{50} and APD_{20}), plateau voltage (V_{PLT}), resting membrane potential (RMP), action potential amplitude (APA) and maximum upstroke velocity (V_{max}). n = 287/201 number of trabeculae/number of patients. Multivariable model, described in methods section, was used to calculate *p*-values. For exact confidence intervals, compare Supplement Table 2. * indicates $p < 0.05$.

Table 2
Interaction between rhythm status and sex on AP parameter.

n = 370/320 for SR n = 287/201 for AF	Scenarios				Comparing scenarios (*p*-values)			
Measurement	Female, sinus rhythm (FSR)	Female, atrial fibrillation (FAF)	Male, sinus rhythm (MSR)	Male, atrial fibrillation (MAF)	FSR vs. FAF	FSR vs. MSR	FAF vs. MAF	MSR vs. MAF
APD_{90} (ms)	314.80 ± 4.19	222.19 ± 4.30	319.91 ± 2.60	216.01 ± 3.34	<0.001	0.301	0.256	<0.001
APD_{50} (ms)	146.87 ± 3.85	101.79 ± 3.98	136.98 ± 2.39	99.19 ± 3.09	<0.001	0.029	0.606	<0.001
APD_{20} (ms)	10.31 ± 1.43	29.00 ± 1.48	6.64 ± 0.89	28.03 ± 1.15	<0.001	0.029	0.603	<0.001
V_{PLT} (mV)	-15.11 ± 0.92	-6.63 ± 0.93	-16.78 ± 0.57	-5.56 ± 0.72	<0.001	0.122	0.366	<0.001
RMP (mV)	-73.16 ± 0.39	-77.27 ± 0.39	-74.58 ± 0.24	-77.57 ± 0.30	<0.001	0.002	0.551	<0.001
APA (mV)	92.8 ± 0.75	100.98 ± 0.77	95.50 ± 0.47	102.20 ± 0.59	<0.001	0.002	0.206	<0.001
V_{max} (V/s)	212.07 ± 7.27	233.1 ± 7.28	234.04 ± 4.50	248.23 ± 5.65	0.041	0.01	0.1	0.049

Values are calculated by mixed-model regression including rhythm state and sex. For details see methods.

4.6. Clinical context: obesity

The mean BMI of patients in our study was 27.5 kg/m² in patients with SR and 28.0 kg/m² in AF group. In contrast to diabetes, we saw effects of obesity. Upstroke velocity, a parameter representing sodium

channel function and highly relevant for conduction, was lower. Conduction was slower in a sheep model of long standing obesity (36 weeks) [41]. The authors described fatty and fibrotic infiltrations of atrial myocardium. Therefore, it remains unclear whether sodium channels contribute to slowed conduction in that model. However, in another

animal model of obesity (mice) sodium current amplitudes were ~ 40% smaller, whereas biophysical properties of sodium channels were not changed. [42] Obesity is associated with AF and patients with higher BMI have shorter atrial ERP [43]. Our results are in line with findings in a recently published animal model of obesity mediated atrial fibrillation, where APA and V_{max} were reduced. [42] Like in our study lower APA and V_{max} was not associated with a change in RMP, suggesting effects mediated at least in part via sodium channels as shown in the mentioned above animal model. [42] Collectively the findings strongly support diminished sodium channel activity in obesity. It would be interesting to see whether obesity affects sodium channel pharmacology, since efficacy of class I but not III drugs to terminate AF is smaller in obese patients [44].

4.7. Clinical context: beta-blocker treatment

Chronic treatment with beta-blockers prolongs APD_{90} in the atrium as well as in the ventricles of rabbits [45,46]. The prolongation in ventricular APD is associated with a prolongation in QTc in rabbits [46] but not in humans [47]. Reason for this species difference remains unclear. In contrast, the prolongation of atrial APD_{90} by chronic treatment with beta-blockers could be confirmed in human atrial cardiomyocytes [48] and in intact tissue (this study). From detailed studies in human atrial cardiomyocytes it can be concluded that posttranslational changes of potassium channel must underlie the effects on APD. [49] The extent of prolongation in APD_{90} fits perfectly to the mentioned above earlier studies [45,46,48]. We assume that beta-blocker mediated prolongation maybe clinically relevant, since it seems to lie in the range that can be expected from pure class III agents obviously able to convert AF and prevent its reoccurrence. In clinical trials on AF, class III dosing was adjusted to limit QT-prolongation to 40 ms. The prototypical pure potassium channel blocker E-4031 prolongs in vitro APD_{90} in both ventricular and atrial tissue from humans [34,50]. Maximum effects of E-4031 on APD_{90} did not differ (~100 ms), however atrial tissue was slightly less sensitive (Supplemental Fig. 6). From a concentration of E-4031 that prolongs APD_{90} in ventricular tissue by 40 ms we have to expect a smaller prolongation in atrial tissue, not larger than seen with chronic beta-blocker treatment (20 ms, Fig. 4). It should be noted that at least in rabbit atria the prolongation in APD_{90} by chronic beta-blocker treatment persisted at high pacing rate, while pure class III agent show less APD prolongation at high rate [51]. Therefore it seems justified to speculate that chronic beta-blocker treatment may be effective in preventing AF reoccurrence [52,53] by prolonging atrial APD_{90} similar to class III compounds. The inability of beta-blockers to convert AF may result from their slow onset of effect (>5 days [46]).

4.8. Clinical context: atrial dilation and left ventricular heart failure

Le Grand et al. described shorter APD_{90} in myocytes isolated from human dilated right atria [54]. Since available routine echocardiography data does reflect right atrial size we are not able to test effect of atrial dilation.

Presence of left ventricular failure has no effect on APD_{90} recorded in isolated myocytes dissociated from right atrial appendages (SR only) [55]. Results were confirmed in a larger study using a multiple linear regression analysis (like in this study) [56]. In stark contrast a more recent study reports larger APD_{90} in right atrial myocytes from patients with reduced EF (both in SR and AF) that was paralleled by a lower expression of two-pore-domain K^+ (K_{2P}) channels [57]. Our data do not support the latter study. We found an association between lower LVEF and lower APA and V_{max}. No data are available from the literature on such an association.

4.9. Clinical context: AF

In tissues from patients with AF we could still detect the association

between QTc and APD_{20} and plateau voltage. In contrast to SR, we saw an association between LVEF and APD_{90} (already shortened). However, all the other associations of AP parameters with age and/or sex were no longer detectable. From this finding we conclude that the impact of AF-related remodeling on such parameters prevails over the gender-related differences in SR (RMP and upstroke velocity).

4.10. Limitations

There are several limitations

1. This is a retrospective analysis. We don't have any follow-up data about occurrence of AF in the SR group.
2. The mean age of our cohort indicates that the majority of female patients has reached menopause. As a result, putative estrogen-mediated effects on electrophysiology may have been lower than in women before menopause. In addition, there is a drop in testosterone levels in elderly man. Thus, in an elderly population the influence of sex hormones is expected to be reduced in both sexes. It would be interesting to extract data from women before menopause. However, our study is not able to give such insights. First, we have much less women than men in our study. Second, women in our study are significantly older. As a result, we could identify only nine women younger than 60 years. This small number does not allow application of our multivariable mixed model regression.
3. We present data from right atrial appendages only. We did not record AP from left atrial tissue. There are substantial differences in electrophysiological properties of different parts of the human heart. [58] We cannot rule out that in other parts than right atrial appendages relevant age-dependent changes in electrophysiology may occur.
4. In AF APD is shortened and loss of shortening of APD upon high stimulation rate is lost. [59] We cannot address this important point since our data are obtained at slow rate only (1 Hz).
5. Only few patients had grossly enlarged left ventricles and reduced ejection fraction, probably hampering to detect an association between heart failure and atrial electrophysiology.
6. We may underestimate effects of drug treatment, since such effects can be restricted to specific pathologies, hampering detection in an unselected population [60].

5. Conclusions

In humans, the increase in AF with age cannot be explained by a shortening in APD. The lower incidence of AF in women associates with less negative RMP and reduced sodium channel function. One may speculate whether pharmacological inhibition of currents regulating RMP could be helpful to prevent reoccurrence of AF.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.yjmcc.2023.01.006.

Data availability statement

The data underlying this article will be shared on reasonable request

to the corresponding author.

Declaration of Competing Interest

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