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Populationsbasierte Ergebnisse zum Zusammenhang zwischen Renin-Angiotensin-Aldosteron System und kardiovaskulären sowie metabolischen Phänotypen

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INHALTSVERZEICHNIS

ABKÜRZUNGSVERZEICHNIS

ACE	Angiotensin-konvertierendes Enzym
ARR	Aldosteron-Renin-Quotient
ATC	Anatomisch–therapeutisch-chemische Klassifikation
ATP III	Adult Treatment Panel III
BMI	Body Mass Index
EDTA	Ethylendiamintetraessigsäure
FMD	Flussvermittelte Vasodilatation
HDL	High density lipoprotein
IDF	International Diabetes Federation
IFCC	International Federation of Clinical Chemistry and Laboratory Medicine
KI	Konfidenzintervall
KORA	Kooperative Gesundheitsforschung in der Region Augsburg
MetS	Metabolisches Syndrom
NCEP	National Cholesterol Education Program
OR	Odds Ratio
PAC	Plasma Aldosteron Konzentration
PAL	Primärer Hyperaldosteronismus
PRC	Plasma Renin Konzentration
RAAS	Renin-Angiotensin-Aldosteron System
SAC	Serum Aldosteron Konzentration
SHIP	Study of Health in Pomerania
WHO	World Health Organization

ABBILDUNGSVERZEICHNIS

TABELLENVERZEICHNIS

1. EINLEITUNG

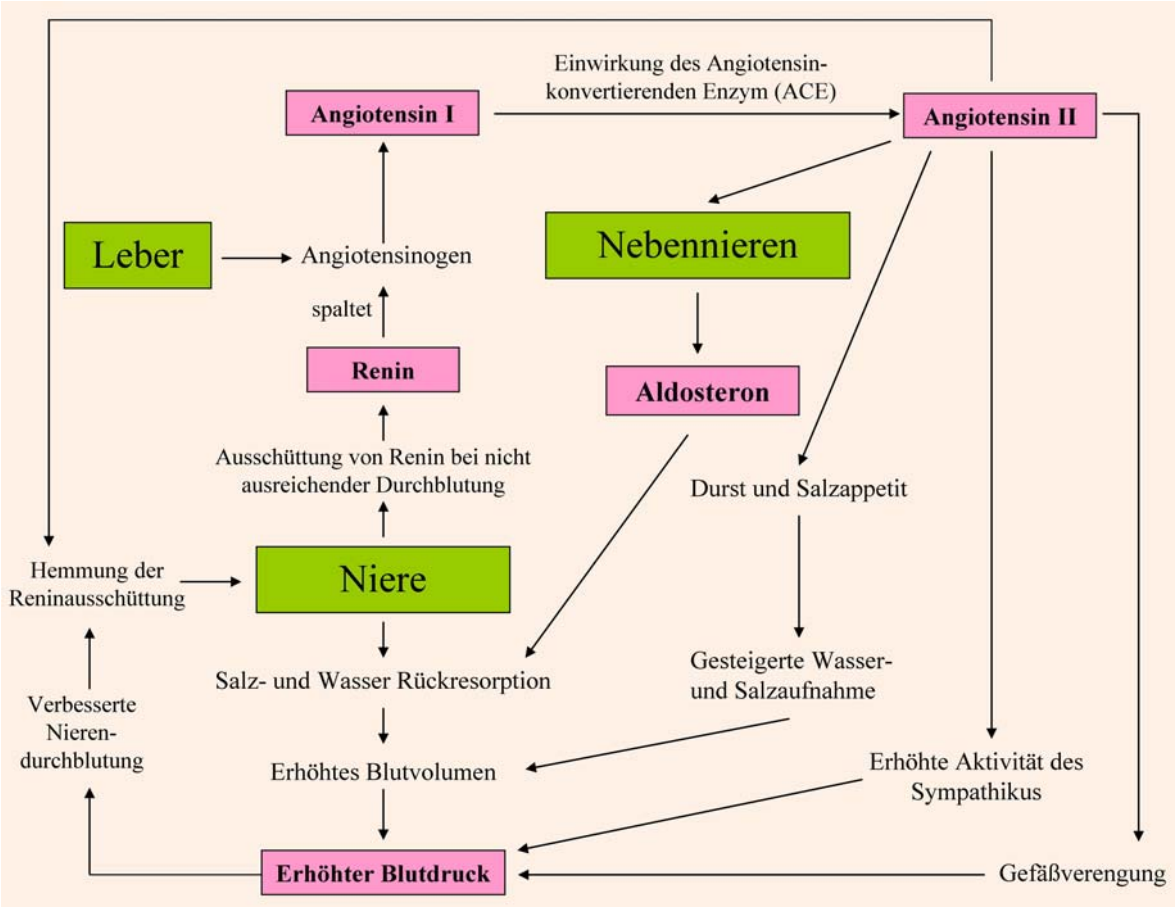
1.1. Das Renin-Angiotensin-Aldosteron System (RAAS)

Arterielle Hypertonie ist eine der häufigsten Erkrankungen bei Erwachsenen in Deutschland und ein wichtiger Prädiktor für kardiovaskuläre Morbidität und Mortalität.[1-3] Laut Bundesgesundheitssurvey von 1998 beträgt ihre Prävalenz etwa 44% bei den Frauen und etwa 51% bei den Männern.[4]

Das RAAS nimmt bei der Blutdruckregulation sowie bei der Aufrechterhaltung des Wasser- und Elektrolythaushalts eine Schlüsselrolle ein.[5] Eine Aktivierung des RAAS kann z. B. bei verminderter Durchblutung des Nierenkörperchens, verringertem Blutvolumen oder Blutdruck aber auch bei einer erniedrigten Natriumkonzentration an der Macula Densa ausgelöst werden.[6] Dies führt zur vermehrten Produktion der Hormone Angiotensin II und Aldosteron. Diese bewirken u. a. eine Vasokonstriktion der Arterien und Arteriolen, eine Verminderung der renalen Natrium- und Wasserausscheidung sowie eine Erhöhung der renalen Kaliumausscheidung. Infolgedessen wird ein Anstieg im Blutdruck erreicht. In Abbildung 1 ist die Funktionsweise des RAAS schematisch dargestellt. Eine ausführliche Beschreibung kann an anderer Stelle nachgelesen werden.[6-8] Neben dem zirkulierenden RAAS existieren in einer Vielzahl von Geweben bzw. Organen lokale RAAS.[6,9] So kann u. a. auch im Herz, den Nieren und dem Gehirn lokal Angiotensin II gebildet werden.[10-13] Lokale RAAS wirken auto- bzw. parakrin und können an pathologischen Entwicklungen beteiligt sein.[14] Beispielsweise wurde bei der Pathogenese der diabetischen Nephropathie eine Beteiligung des intra-renalen RAAS festgestellt.[15]

Aufgrund seiner blutdruckregulierenden Funktion ist das RAAS ein wichtiges Ziel für die antihypertensive Therapie.[7] So werden ACE Hemmer und Angiotensin II Rezeptor Antagonisten regulär zur Behandlung der Hypertonie eingesetzt, mit dem Ziel das RAAS zu blockieren.[7,16] Auch die Mineralokortikoid Rezeptor Anatagonisten Spironolakton und Eplerenon haben sich in der antihypertensiven Therapie bewiesen.[6,7] Sie werden ebenso wie die ACE Hemmer und Angiotensin II Rezeptor Blocker auch bei weiteren kardiovaskulären Erkrankungen, einschließlich der diabetischen Nephropathie, der links ventrikulären Dysfunktion und der chronisch systolischen Herzinsuffizienz, angewandt.[6,7,17] Seit 2007 sind außerdem direkte Reninhemmer zur Behandlung der Hypertonie zugelassen.[17]

Abbildung 1. Schematische Darstellung der Funktionsweise des RAAS



RAAS, Renin-Angiotensin-Aldosteron System

Ein systemischer Blutdruckabfall aber auch ein Natriummangel, eine erhöhte Kaliumkonzentration oder eine Aktivierung des sympathischen Nervensystems stimulieren die Sekretion des Enzyms Renin in den Zellen des juxtaglomerulären Apparates in der Niere. Unter Einwirkung von Renin wird das in der Leber gebildete Prohormon Angiotensinogen in das biologisch inaktive Angiotensin I umgewandelt. Unter Einwirkung des Angiotensin-konvertierenden Enzyms (ACE) entsteht aus dem Angiotensin I das biologisch aktive Hormon Angiotensin II. Angiotensin II wiederum bewirkt eine Engstellung der Gefäße, stimuliert die Aldosteronproduktion in der Zona Glomerulosa der Nebennierenrinde und löst ein Durstgefühl und Salzappetit aus. Das Steroidhormon Aldosteron wirkt am distalen Tubulus der Niere und bewirkt eine Natrium- und Wasserresorption. Die Engstellung der Gefäße sowie die Erhöhung des extrazellulären Volumens durch Natrium und Wasserrückresorption führen zu einer Erhöhung des Blutdrucks. Angiotensin II, Aldosteron aber auch der höhere Blutdruck wirken direkt hemmend auf die Reninsekretion.[6-8] Damit schließt sich der Regelkreis.

1.2. Störungen des RAAS

Das RAAS kann durch verschiedene Ursachen gestört werden und dadurch zum Auslöser einer arteriellen Hypertonie werden. Eine Störung des RAAS kann durch eine Reihe seltener genetischer Erkrankungen wie z. B. dem Liddle Syndrom, der Glukokortikoid-resistenz oder dem 11-beta-Hydroxylase Mangel hervorgerufen werden.[18,19] Diese Erkrankungen bewirken niedrige Renin und Aldosteron Serumkonzentrationen. Andere Störungen die eine endokrin bedingte arterielle Hypertonie verursachen, gehen mit einer erhöhten Aldosteronkonzentration einher. So wird bei der Nierenarterienstenose, aufgrund einer Verengung der Nierenarterien, das Organ nicht ausreichend durchblutet und die Reninproduktion chronisch stimuliert. Die betroffenen Patienten weisen pathologisch erhöhte Aldosteronkonzentrationen auf und entwickeln eine Hypertonie, die auch als renovaskuläre Hypertonie bezeichnet wird.[20,21] Im Unterschied dazu weisen Patienten mit primärem Hyperaldosteronismus (PAL) niedrige Renin- und erhöhte Aldosteron-konzentrationen auf.[5,22] Beim PAL wird eine autonome Aldosteronproduktion der Nebenniere beobachtet.[5,22] PAL wird im Großteil der Fälle durch Aldosteron-produzierende Adenome in der Nebenniere bzw. uni- oder bilaterale Hyperplasie der Nebennieren verursacht.[23] Auch monogenetische Formen des PAL sind bekannt (familiärer Hyperaldosteronismus I-III), aber äußerst selten.[24]

1.2.1. Herleitung der 1. Fragestellung

Das Krankheitsbild des PAL (auch Conn Syndrom genannt) wurde erstmalig in den 50er Jahren des 20. Jahrhunderts von Dr. J.W. Conn beschrieben.[25] Nachdem es lange Zeit für eine seltene Erkrankung gehalten wurde, nahm die Identifikation von Patienten mit PAL in den 90er Jahren des 20. Jahrhunderts deutlich zu.[23] Dies ist einerseits auf die verbesserten laborchemischen Nachweismethoden für Renin und Aldosteron und der damit verbundenen Einführung des Aldosteron-Renin-Quotienten (ARR) als Screeningtest zurückzuführen. Andererseits erkannte man, dass ein Großteil der Patienten nicht wie ursprünglich angenommen hypokaliämisch, sondern normokaliämisch ist.[23] Heute wird angenommen, dass der PAL die häufigste Form der sekundären Hypertonie ist, die exakte Prävalenz des PAL ist allerdings unbekannt. Ein Review[26] aus dem Jahr 2008 berichtet Prävalenzen zwischen 1-13% bei hypertensiven Patienten in der Primärversorgung.

Für Patienten mit PAL ist eine frühzeitige Diagnose essentiell, da es unbehandelt zu einer Reihe von Endorganschäden beiträgt. So konnte gezeigt werden, dass Patienten

mit PAL ein höheres Risiko für kardiovaskuläre Ereignisse, einschließlich Myokardinfarkt, Schlaganfall und Vorhofflimmern aufweisen als Patienten mit essentieller Hypertonie.[27] Die Endocrine Society[22] empfiehlt daher Screeningtests für PAL in Patientengruppen mit hoher Prävalenz, u. a. bei therapieresistenter Hypertonie, Hypertonie Grad 2 und Grad 3, Hypokaliämie oder beim Auftreten eines adrenalen Inzidentaloms. Als Screeningtest wird der ARR empfohlen,[22] der einen relativen Aldosteronüberschuss im Vergleich zum Renin anzeigen kann. Aufgrund seiner eingeschränkten Sensitivität und Spezifität ist ein alleiniger positiver ARR Screeningtest nicht mit einer Diagnose gleichzusetzen.[28] Positive ARR Screeningergebnisse müssen mithilfe von Bestätigungstests, z. B. dem intravenösen oder dem oralen Salzbelastungstest oder dem Fludrocortison Test, verifiziert werden.[22,29]

Der ARR ist als Screeningtest für den PAL international weitgehend anerkannt,[30] einen einheitlichen Grenzwert für ein positives Screeningergebnis gibt es allerdings nicht.[26] Aufgrund unterschiedlicher Labormethoden zur Bestimmung von Aldosteron und Renin[31,32] sowie unterschiedlicher Blutabnahmebedingungen[28,33,34] ist dies auch kaum möglich. So kann Renin als Aktivität oder Konzentration gemessen werden,[35] weiterhin beeinflussen die Tageszeit,[28,34] der aktuelle Salzkonsum,[36] die Einnahme unterschiedlicher Medikamente[22] aber auch das Alter[37] und Geschlecht[38] die Aldosteron- und Reninkonzentration.

Die in diversen Studien ermittelten ARR Grenzwerte für das PAL Screening unterscheiden sich daher deutlich.[26] Diese Grenzwerte wurden überwiegend anhand von Vergleichen des ARR von Patienten mit und ohne PAL ermittelt. Eine andere Möglichkeit um zwischen einem physiologischen und pathophysiologischen ARR zu unterscheiden liefern Referenzbereiche. Ein Referenzbereich ist in der Regel definiert als zentrales 95% Intervall der Verteilung eines Messwertes in einer gesunden Referenzpopulation.[39] Die Werte unterhalb des 2.5% oder oberhalb des 97.5% Perzentils der Verteilung werden als erniedrigt oder erhöht angesehen.[40] Ein Referenzbereich bietet dem Kliniker eine Orientierung über die Lage eines Messwertes im Bezug zu einer gesunden Population, liefert aber keine Diagnose. So haben Individuen mit Messwerten außerhalb des Referenzbereichs eine höhere Wahrscheinlichkeit nicht gesund zu sein als Individuen innerhalb des Referenzbereichs. Messwerte außerhalb des Referenzbereichs sind aber nicht mit Krankheit und Messwerte innerhalb des Referenzbereichs nicht mit Gesundheit gleichzusetzen.[40]

Neben dem Referenzbereich für den ARR werden auch Referenzbereiche für Aldosteron und Renin benötigt. So können niedrige Aldosteronkonzentrationen u. a. auf eine primäre Nebennierenrindeninsuffizienz oder eine Hypophyseninsuffizienz hindeuten,[41,42] bzw. niedrige Reninkonzentrationen auf ein Liddle- oder Gordon Syndrom.[18,19] Da Referenzbereiche methodenabhängig sind, werden sie in der Regel von den Herstellern der Aldosteron und Renin Assays erstellt. Diese vom Hersteller angegebenen Referenzbereiche werden allerdings oft an kleinen, nicht repräsentativen und nicht ausreichend phänotypisierten Stichproben ermittelt. Daher empfiehlt die International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) die Ermittlung von laborspezifischen Referenzbereichen anhand der lokalen Population.[39,40] Ziel der vorliegenden Analyse war die Verteilung der Aldosteronkonzentration im Plasma (PAC), der Reninkonzentration im Plasma (PRC) und des ARR in einer gesunden Population darzustellen sowie alters- und geschlechtsspezifische Referenzwerte zu ermitteln.

1.3. Das RAAS und kardiovaskuläre Erkrankungen

Eine Beteiligung des RAAS bei der Pathogenese kardiovaskulärer Erkrankungen wurde in einer Vielzahl von Studien belegt.[43-50] So wurde u. a. nachgewiesen, dass Angiotensin II inflammatorische Prozesse, Zellwachstum und Apoptose stimuliert[44,45] und zum kardialen Remodeling beiträgt.[46] Auch Aldosteron nimmt eine bedeutende Rolle bei der Pathogenese kardiovaskulärer Erkrankungen ein, da es prooxidative, proinflammatorische und profibrotische Effekte ausübt.[43,51] Die exakten Mechanismen die diesen Effekten unterliegen, sind bisher nur teilweise geklärt.[14,52,53]

In epidemiologischen Studien wurde bei Patienten mit PAL ein Zusammenhang zwischen der PAC und dem linksventrikulären Masseindex nachgewiesen, der weitgehend unabhängig vom Blutdruck ist.[47-50] Weiterhin wurde bei Patienten mit PAL eine höhere Intima Media Dicke und eine höhere Pulswellengeschwindigkeit als bei Patienten mit essentieller Hypertonie festgestellt.[54] Auch bei Individuen ohne PAL wurden schädigende Effekte des Aldosterons auf das kardiovaskuläre System beschrieben.[55,56] So wiesen Ramachandran und Kollegen[55] anhand der Daten der populationsbasierten Framingham Heart Study nach, dass Individuen mit einer Aldosteron Serumkonzentration (SAC) im oberen Normalbereich im Vergleich zu Individuen mit einer SAC im unteren Normalbereich ein erhöhtes Risiko für eine inzidente Hypertonie aufweisen. In einer

weiteren Untersuchung[56] der Framingham Heart Study wurde eine Assoziation zwischen der SAC und dem links ventrikulären konzentrischen Remodeling bei Frauen beobachtet.

1.3.1. Herleitung der 2. Fragestellung

Das gesunde vaskuläre Endothel übernimmt eine Vielzahl von Funktionen im Organismus. Es dient der Regulierung des Gefäßtonus und reagiert auf physische und chemische Signale aus dem Blutkompartiment indem es Faktoren produziert, die den Gefäßtonus, die Zelladhäsion, die Thromboresistenz, die Proliferation von glatten Muskelzellen und die Inflammation der Gefäßwand regulieren.[57] Kardiovaskuläre Risikofaktoren wie Diabetes Mellitus oder Übergewicht können die Endothelfunktion schädigen.[58] Eine Verminderung der Endothelfunktion ist ein erstes Anzeichen für Schäden an der Gefäßwand. Sie wird daher als Marker für die subklinische Atherosklerose angesehen,[57-59] stellt jedoch keine eigenständige Erkrankung dar.

Die Endothelfunktion kann anhand der Messung der Flussvermittelten Vasodilatation (FMD) abgeschätzt werden.[60] Bei der FMD wird durch Kompression des Oberarms eine funktionelle Ischämie in der Mikrozirkulation erzeugt. Nach Aufhebung der passageren Ischämie kommt es in Abhängigkeit von der Endothelreagibilität zu einer Steigerung der Blutflussgeschwindigkeit in der Armarterie, was zu einer Zunahme des Gefäßdiameters führt. Die Änderung des Gefäßdiameters, die per Sonographie gemessen werden kann, wird als FMD bezeichnet.[60]

In früheren Studien konnte gezeigt werden, dass die FMD Messung neben den traditionellen Risikofaktoren einen zusätzlichen Prädiktor für kardiovaskuläre Ereignisse darstellt.[61,62] Gleichzeitig wurde beobachtet, dass eine chronisch erhöhte PAC, wie beim unbehandelten PAL, mit einer endothelialen Dysfunktion einhergeht.[63,64] Patienten mit PAL weisen signifikant niedrigere FMD Werte als Patienten ohne PAL auf. Nach medikamentöser oder chirurgischer Therapie des PAL ist wiederum eine Verbesserung der FMD zu erkennen.[63,64] Während die aufgezeigten Studien auf kleinen PAL Patientenkollektiven basieren, ist über die Assoziation zwischen der PAC bzw. dem ARR und der FMD in der Allgemeinbevölkerung nur wenig bekannt.[65]

Ziel der vorgestellten Studie war es herauszufinden, ob die PAC oder der ARR in einer großen Studie basierend auf der Normalbevölkerung mit einer eingeschränkten FMD einhergehen. Zusätzlich sollte analysiert werden, ob etwaige Zusammenhänge auch innerhalb des studienspezifischen Referenzbereichs für die PAC oder den ARR auftreten.

1.4. Das RAAS und metabolische Erkrankungen

Neben der Vielzahl an Studien die Effekte des RAAS auf das kardiovaskuläre System beschreiben, mehren sich in den letzten Jahren Untersuchungen, die den Einfluss des RAAS auf den Metabolismus analysieren (für ein Review siehe[66]). Das RAAS wird dabei im Zusammenhang mit pathologischen Veränderungen des Glukosemetabolismus, des Fettstoffwechsels und der Insulinresistenz gesehen.[66] So wurde u. a. erkannt, dass Angiotensin II in Fettgewebe lokal synthetisiert werden kann und dort das Adipozytenwachstum stimuliert.[66,67] Des Weiteren wurden Assoziationen zwischen Aldosteron und metabolischen Veränderungen beschrieben.[68-71] Beispielsweise wurden schädigende Effekte ausgehend vom Aldosteron auf die Funktion der insulin-produzierenden ß-Zellen im Pankreas beschrieben.[68,69] Andere Ergebnisse aus Zellkulturstudien und Tiermodellen[72-74] weisen auf einen komplexen Austausch zwischen dem Fettgewebe und den Nebennieren hin. Diese Untersuchungen legen nahe, dass Übergewicht bzw. Adipositas mit einer erhöhten Aldosteronproduktion einhergeht und diese wiederum die Entwicklung von metabolischen Störungen hervorrufen oder perpetuieren kann.[70]

1.4.1. Herleitung der 3. Fragestellung

Als Metabolisches Syndrom (MetS) wird das gemeinsame Auftreten von multiplen metabolischen Veränderungen bezeichnet.[75] Zur Definition des MetS werden die kardiovaskulären Risikofaktoren viszerale Adipositas, erhöhter Blutzucker, erhöhte Triglyceride, erniedrigtes HDL-Cholesterol sowie erhöhter Blutdruck herangezogen.[75] Bei Vorliegen von mindestens drei dieser Komponenten kann ein MetS festgestellt werden. Bisher hat sich jedoch keine einheitliche Definition hinsichtlich der Auswahl der Komponenten bzw. ihrer Grenzwerte durchgesetzt.[75] In Tabelle 1 sind drei wichtige Definitionen des MetS zusammengestellt.

In prospektiven Studien wurden Assoziationen zwischen dem MetS und inzidenter kardiovaskulärer Morbidität und Mortalität nachgewiesen.[79,80] Ob das MetS jedoch die Risikoprädiktion für kardiovaskuläre Erkrankungen gegenüber seinen Einzelkomponenten verbessert, ist umstritten.[81,82]

Tabelle 1. Definition des Metabolischen Syndroms nach World Health Organization (WHO), National Cholesterol Education Program Expert Panel (NCEP) und International Diabetes Federation (IDF).

WHO (1998)[76]	**NCEP-ATP III (2002)**[77]	**IDF (2005)**[78]
Mindestens eine der folgenden Komponenten: - Glukoseintoleranz, - Gestörte Glukosetoleranz oder diagnostizierter Diabetes Mellitus - Insulinresistenz Zusätzlich mindestens zwei der folgenden Komponenten: - Eingeschränkte Glukoseregulation oder diagnostizierter Diabetes Mellitus - Insulinresistenz - Erhöhter arterieller Blutdruck (≥160/90 mmHg) - Erhöhte Triglyceride (≥1,7 mmol/l) - niedriges HDL-Cholesterol (Männer <0,9 mmol/l; Frauen <1,0 mmol/l) - Erhöhtes Taille-Hüft-Verhältnis (Männer >0,90; Frauen >0,85) oder BMI >30kg/m^2 - Mikroalbuminurie (Albumin im Urin ≥20 µg/min oder Albumin-Kreatinin-Verhältnis ≥20 mg/g)	Mindestens drei der folgenden Komponenten: - Erhöhte Nüchternglukose (≥110 mg/dl) - Erhöhter arterieller Blutdruck (≥130/85 mmHg) - Erhöhte Triglyceride (≥150 mg/dl) - Niedriges HDL-Cholesterol (Männer <40 mg/dl; Frauen <50 mg/dl) - Erhöhter Taillenumfang (Männer >102 cm; Frauen >88 cm)	Mindestens: - Erhöhter Taillenumfang (Ethnizitätsspezifisch) oder BMI >30 kg/m^2 Zusätzlich mindestens zwei der folgenden Komponenten: - Erhöhte Nüchternglukose (≥5,6mmol/l) oder diagnostizierter Typ 2 Diabetes Mellitus - Erhöhter arterieller Blutdruck (≥130/85 mmHg) - Erhöhte Triglyceride (>150 mg/dl) oder Einnahme von lipidsenkender Medikation - Niedriges HDL-Cholesterol (Männer <40mg/dl; Frauen <50 mg/dl)

BMI, body mass index; HDL-Cholesterol, High density lipoprotein Cholesterol

Verschiedene epidemiologische Studien legen Nahe, dass Aldosteron mit dem Auftreten eines MetS bzw. metabolischer Veränderungen assoziiert ist.[83-91] So konnte nachgewiesen werden, dass Patienten mit PAL eine höhere Prävalenz des MetS[83,84] sowie des Diabetes Mellitus[85] aufweisen als Patienten mit essentieller Hypertonie. In einer weiteren Studie wurde ein Zusammenhang zwischen PAL und Insulinresistenz berichtet.[90] Zusätzlich zeigte sich, dass nach Therapie des PAL eine Verbesserung der Insulinsensitivität erreicht wird.[90] Auch bei Individuen ohne PAL wurden Assoziationen zwischen der PAC und metabolischen Veränderungen berichtet. So wurden positive Korrelationen zwischen der PAC und dem Taillenumfang[84,91] und inverse Korrelationen zwischen der PAC und dem HDL-Cholesterol[84,89] sowie der Insulinsensitivität nachgewiesen.[87,88] Ziel der vorgestellten Analyse war es die Assoziation zwischen der PAC und dem MetS sowie seinen Einzelkomponenten in zwei großen deutschen Kohortenstudien zu prüfen.

2. METHODEN

2.1. Studiendesign und Studienpopulationen

Die der Arbeit zugrundeliegenden Daten stammen aus der jeweils ersten Follow-up Untersuchung der Study of Health in Pomerania (SHIP-1) und dem F4 Survey der Kooperativen Gesundheitsforschung in der Region Augsburg (KORA F4).

SHIP ist eine longitudinale, populationsbasierte Studie in Vorpommern. Die zwei wesentlichen Ziele der Studie sind die Ermittlung der Prävalenz und Inzidenz von subklinischen und klinischen Erkrankungen sowie ihrer Risikofaktoren und die Erforschung von Assoziationen zwischen Risikofaktoren und Erkrankungen.[92,93] Basierend auf Melderegisterdaten wurde in einem zweistufigen Prozess eine randomisierte, repräsentative Stichprobe der 20-79-jährigen Einwohner in der Region West Vorpommern gezogen. Von den 7008 gezogenen Männern und Frauen nahmen 4308 Probanden an der Basisuntersuchung (SHIP-0) zwischen 1997 und 2001 teil. Alle Probanden wurden erneut zur 5-Jahres Follow-up Untersuchung (SHIP-1) eingeladen. Von ihnen nahmen 3300 Probanden an den Untersuchungen zwischen 2003 und 2006 teil. In der Basis– sowie der ersten Follow-up Studie wurde allen Probanden ein umfangreiches medizinisches Untersuchungsprogramm angeboten, einschließlich anthropometrischer, sonographischer und zahnärztlicher Untersuchungen, Blut- und Urinuntersuchungen sowie einem detaillierten persönlichen Interview zur Krankengeschichte und zum Lebensstil. Details zur Stichprobenziehung und zum Untersuchungsprogramm finden sich an anderer Stelle.[92,93]

KORA ist eine Forschungsplattform von der eine Reihe populationsbasierter Studien initiiert wurde. Thematische Schwerpunkte der Studien bilden die Erforschung von Risikofaktoren kardiovaskulärer und metabolischer Erkrankungen.[94] Der KORA F4 Survey ist das erste 5-Jahres Follow-up des KORA S4 Surveys. Für den KORA S4 Survey wurde, basierend auf Melderegisterdaten, in einem zweistufigen Prozess eine randomisierte, repräsentative Stichprobe der 25-74-jährigen Einwohner der Stadt Augsburg und 16 umliegender Gemeinden gezogen. Von den 6640 gezogenen Männern und Frauen nahmen 4261 Probanden an der Basisuntersuchung zwischen 1999 und 2001 teil. Alle Probanden wurden erneut zur 5-Jahres Follow-up Untersuchung (KORA F4) eingeladen. Von ihnen nahmen 3080 an den Untersuchungen zwischen 2006-2008 teil. Wie auch in SHIP wurde den KORA Probanden ein umfangreiches medizinisches Untersuchungsprogramm

angeboten. Details zur Stichprobenziehung und zum Untersuchungsprogramm finden sich an anderer Stelle.[94]

2.2. Datenerhebung und Labormethodik

2.2.1. Datenerhebung

In SHIP-1 sowie in KORA F4 wurden sozio-demographische und verhaltensorientierte Charakteristika der Studienteilnehmer in standardisierten, persönlichen Interviews erfragt. Weiterhin wurden die Probanden gebeten alle Arzneimittel zur Untersuchung mitzubringen, die sie an den vorausgehenden sieben Tagen eingenommen hatten. Den Präparaten wurde anhand der amtlichen deutschen Fassung der anatomisch-therapeutisch-chemischen Klassifikation (ATC)[95] der Wirkstoffname zugeordnet. Im Rahmen der anthropometrischen Messungen wurden Größe und Gewicht der Probanden mit geeichten Geräten gemessen. Der Taillenumfang wurde mit einem unelastischen Maßband an der schmalsten Stelle zwischen der letzten Rippe und der höchsten Stelle des Darmbeinkammes gemessen. Die Messung des systolischen und diastolischen Blutdrucks erfolgte im Sitzen am rechten Arm des Probanden. Nach einer Ruhephase von mindestens fünf Minuten erfolgten drei Blutdruckmessungen im Abstand von jeweils drei Minuten mit einem automatischen, oszillometrischen Messgerät (HEM-705CP, OMRON Corporation, Tokyo, Japan). Für statistische Analysen wurden die Mittelwerte des systolischen und diastolischen Blutdrucks aus zweiter und dritter Messung verwendet.

2.2.2. Labormethodik

In SHIP-1 erfolgte die Blutabnahme aus der Kubitalvene ganztägig an überwiegend nicht-nüchternen Probanden in Rückenlage. Die PAC und PRC wurden mit radioimmunometrischen Assays im EDTA-Plasma gemessen (PAC: Coat-A-Count Aldosterone, Siemens Healthcare Diagnostics GmbH, Eschborn, Germany, PRC: Renin III Generation, Cisbio Bioassay, Bagnols-sur- Cèze Cedex, France). Triglyceride und Glukose wurden im Serum mit enzymatischen Methoden gemessen (Hitachi 717, Roche Diagnostics GmbH, Mannheim, Germany). Das HDL-Cholesterol wurde im Serum mittels Lipoproteinelektrophorese (HELENA SAS-3 System; Helena 7 BioSciences Europe, Tyne & Wear, U.K.) gemessen. Kalium wurde im Serum mittels indirekter Potentiometrie mit Ionen-selektiven Elektroden gemessen (QuikLYTE, Dade Behring, Eschborn, Germany).

Kreatinin wurde im Serum mittels einer modifizierten Jaffé Methode gemessen (Hitachi 717, Roche Diagnostics GmbH, Mannheim, Germany).

In KORA F4 erfolgte die Blutabnahme aus der Kubitalvene am Morgen an nüchternen, sitzenden Probanden. Die PAC wurde im EDTA Plasma mit einem selbst entwickelten immunofluoreszenz Assay gemessen.[96] Triglyceride, Glukose und HDL-Cholesterol wurden im Serum mit enzymatischen Methoden gemessen (Triglyceride und HDL-Cholesterol: Dimension RxL, Siemens Healthcare Diagnostics, Eschborn, Germany; Glukose: Hitachi 717, Roche Diagnostics GmbH, Mannheim, Germany). Kalium wurde im Serum mittels indirekter Potentiometrie mit Ionen-selektiven Elektroden gemessen (QuikLYTE, Dade Behring, Eschborn, Germany).

2.3. Selektion der Referenzpopulation

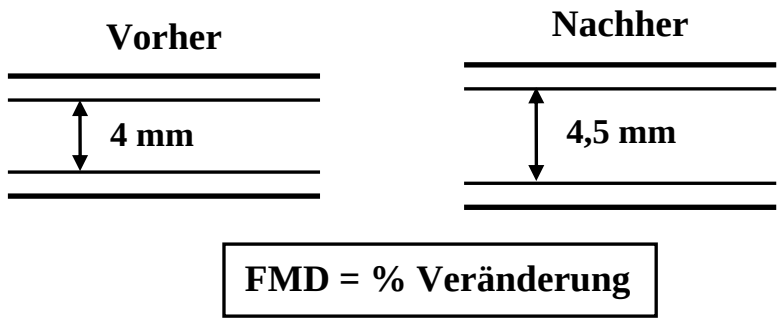
Referenzwerte für die PAC, PRC und den ARR wurden anhand der SHIP-1 Daten erstellt. Zu diesem Zweck wurde eine Subgruppe bestehend aus 1347 gesunden Referenzindividuen ausgewählt. Probanden wurden als gesund angesehen, wenn sie keine potentielle Störung des RAAS aufwiesen. Von der gesamten SHIP-1 Studienpopulation wurden, neben den Probanden ohne PAC und PRC Messung, alle Probanden ausgeschlossen, die eine extrem hohe PAC oder PRC hatten (PAC oder PRC >1000 ng/l) und einen sekundären Hyperaldosteronismus vermuten ließen, sowie Probanden mit Hypokaliämie (Kaliumkonzentration im Serum <3,5 mmol/l), Hypertonie (systolischer Blutdruck ≥140 mmHg oder diastolischer Blutdruck ≥90 mmHg) oder Niereninsuffizienz (geschätzte glomeruläre Filtrationsrate nach Cockcroft-Gault <50 ml/min). Weiterhin wurden alle Probanden, die Medikamente mit Auswirkung auf das RAAS einnahmen (ATC Klassen C02, C03, C07, C08, C09), schwangere Frauen und Probanden älter als 74 Jahre (aufgrund ihrer geringen Anzahl), ausgeschlossen.

2.4. Messung der FMD

Allen SHIP-1 Probanden wurde eine FMD Messung angeboten, 1692 Probanden nahmen an der Untersuchung teil. Die FMD Messung fand am Tag der SHIP Hauptuntersuchung oder an einem späteren Termin statt. Im Median vergingen 16,5 Tage (1. und 3. Quartil: 0 und 75 Tage) zwischen Hauptuntersuchung und FMD Messung. Bei der FMD Messung wurde zunächst in Ruhe der Durchmesser der Arteria Brachialis ca. 3-7 cm oberhalb des Ellenbogens sonographisch vermessen. Der Proband befand sich dabei in Rückenlage. Die

Untersuchung fand am rechten Arm statt. Die Messung wurde mit Hilfe eines Linear-Array-Schallkopfes (Cypress, Siemens AG, Erlangen, Germany) durchgeführt. Anschließend wurde eine Blutdruckmanschette auf einen Druck von 220 mmHg aufgeblasen und für fünf Minuten am Arm des Probanden belassen. Die Kompression im Oberarm erzeugt eine vorübergehende Ischämie. Nach Ablassen des Drucks aus der Blutdruckmanschette kommt es zu einer Steigerung der Blutflussgeschwindigkeit in der Arteria Brachialis, die zu einer Zunahme des Gefäßdurchmessers führt. Eine Minute nach Ablassen des Drucks fand eine erneute sonographische Messung des Arteriendurchmessers statt. Die FMD gibt die Veränderung im Arteriendurchmesser zwischen Ruhezustand und nach Ischämie wieder. Sie kann als absolute oder, wie in der vorliegenden Studie, als prozentuale Differenz angegeben werden (Abbildung 2). Details zur Qualitätssicherung der FMD Messung sind an anderer Stelle nachzulesen.[97]

Abbildung 2. Schematische Darstellung des Durchmessers der Arteria Brachialis vor und nach Erzeugung einer passageren Ischämie.



2.4.1. Zusätzlich berücksichtigte Kovariaten

Die Selektion der Kovariaten wird im Abschnitt 2.6. beschrieben. In der Analyse wurden folgende Einflussgrößen berücksichtigt:

- Geschlecht (männlich, weiblich)
- Alter (in Jahren)
- Systolischer Blutdruck (in mmHg)
- Diastolischer Blutdruck (in mmHg)
- Taillenumfang (in cm)
- Aktueller Rauchstatus (aktueller Raucher, aktueller Nichtraucher)
- Diabetes Mellitus (ja, nein)
- Sport (ja, nein)
- Zeit zwischen Blutabnahme und FMD Messung (in Tagen)

2.5. Definition des MetS

Eine allgemeingültige, standardisierte Definition für das MetS gibt es bisher nicht.[75] In der vorliegenden Studie wurde die Definition von Alberti und Kollegen[75] aus dem „Joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention, National Heart, Lung, and Blood Institute, American Heart Association, World Heart Federation, International Atherosclerosis Society, and International Association for the Study of Obesity" verwendet. Das MetS wird nach dieser Definition festgestellt, wenn eine Person mindestens drei der folgenden fünf Komponenten erfüllt: (1) erhöhter Taillenumfang, (2) erhöhte Glukose, (3) niedriges HDL-Cholesterol, (4) erhöhte Triglyceride und (5) erhöhter Blutdruck. Die jeweiligen Schwellenwerte zur Feststellung der Komponenten des MetS konnten in KORA F4 unverändert angewandt werden, da alle Blutproben von nüchternen Probanden stammen. Da die SHIP-1 Blutproben von überwiegend nicht nüchternen Probanden stammen, wurden die Schwellenwerte für erhöhte Glukose und erhöhte Triglyceride leicht modifiziert (Tabelle 1). Dabei wurden Schwellenwerte aus einer Studie von Lidfeldt und Kollegen[98] übernommen, die anhand von 6805 Blutproben nicht nüchterner, schwedischer Frauen Risikofaktoren für kardiovaskuläre Erkrankungen und Diabetes Mellitus ermittelten.

Tabelle 2. Definition des Metabolischen Syndroms (MetS) in SHIP-1 und KORA F4

Komponenten des MetS*	**SHIP-1**	**KORA F4**
Erhöhter Taillenumfang	Männer ≥94 cm; Frauen ≥80 cm	
Erhöhte Glukose	Glukose, nicht nüchtern: ≥8 mmol/l oder antidiabetische Medikation (ATC A10A, A10B)	Glukose, nüchtern: ≥5,55 mmol/l oder antidiabetische Medikation (ATC A10A, A10B)
Niedriges HDL-Cholesterol	Männer <1,0 mmol/l; Frauen <1,3 mmol/l oder lipidsenkende Medikation (ATC C10AB, A10AD)	
Erhöhte Triglyceride	Triglyceride, nicht nüchtern: ≥2,3 mmol/l oder lipidsenkende Medikation (ATC C10AB, A10AD)	Triglyceride, nüchtern: ≥1,7 mmol/l oder lipidsenkende Medikation (ATC C10AB, A10AD)
Erhöhter Blutdruck	Systolischer oder diastolischer Blutdruck ≥130/85 mmHg oder selbstberichtete Einnahme von antihypertensiver Medikation† bei Patienten mit selbstberichteter Hypertonie	

ATC, anatomisch-therapeutisch-chemische Klassifikation; HDL-Cholesterol, High density lipoprotein Cholesterol; KORA, Kooperative Gesundheitsforschung in der Region Augsburg; SHIP, Study of Health in Pomerania. *Das MetS ist definiert als Präsenz von mindestens drei der fünf Komponenten aus der Tabelle. †Eingenommene Präparate wurden basierend auf den Empfehlungen der Deutschen Hypertonieliga [SHIP:[99], KORA:[100]] als antihypertensive Medikation definiert.

2.5.1. Zusätzlich berücksichtigte Kovariaten

Die Selektion der Kovariaten wird im Abschnitt 2.6. beschrieben. In der Analyse wurden folgende Einflussgrößen berücksichtigt:

- Geschlecht (männlich, weiblich)
- Alter (in Jahren)
- Kaliumkonzentration im Serum (mmol/l)
- Einnahme von Östrogenpräparaten (ATC G03A, G03C, G03D, G03F) bei Frauen (ja, nein)
- Einnahme von Medikamenten (ja, nein) die die PAC erhöhen (Diuretika ATC C03); nicht in Modellen mit erhöhtem Blutdruck oder MetS als abhängiger Variable.
- Einnahme von Medikamenten (ja, nein) die die PAC erniedrigen (Betablocker ATC C07 und weitere Antiadrenergika ATC C02; Kalziumkanalblocker ATC C08; ACE Hemmer ATC C09A, C09B; Angiotensin II Rezeptor Antagonisten ATC C09C, C09D); nicht in Modellen mit erhöhtem Blutdruck oder MetS als abhängiger Variable.

2.6. Statistische Methoden

Zur Beschreibung der Studienpopulationen wurden Verfahren der deskriptiven Statistik angewandt. Gruppenunterschiede wurden mit dem Kruskal-Wallis Test bei kontinuierlichen bzw. dem Chi-Quadrat Test bei nominalskalierten Variablen auf statistische Signifikanz getestet. Wenn nicht anderweitig gekennzeichnet, wurde ein p-Wert <0,05 als statistisch signifikant angesehen.

Als Referenzbereich wurde der zentrale 95% Bereich der Verteilung der PAC, PRC und des ARR definiert, das 2,5% sowie das 97,5% Perzentil wurden als unterer bzw. oberer Referenzwert festgelegt. Die Berechung der Referenzbereiche wurde getrennt für 25-54-jährige und 55-74-jährige Probanden sowie für die gesamte Population durchgeführt. Um repräsentative Aussagen für die Studienregion treffen zu können, wurden alle Daten für eine Nicht-Teilnahme an der Basisuntersuchung sowie für ein Ausscheiden zwischen Basis- und erster Follow-up Untersuchung gewichtet. Die Gewichte basieren auf sozio-demographischen und gesundheitsbezogenen Daten.

Zur Beurteilung des Zusammenhangs zwischen der PAC oder dem ARR und der FMD wurden ordinale[101,102] sowie quantile[103,104] Regressionsmodelle konstruiert. Da keine standardisierten Grenzwerte für eine Abgrenzung zwischen normalen und niedrigen FMD Werten existieren,[105] wurde die FMD im ordinalen Regressionsmodell verteilungsbasiert in alters- und geschlechtsspezifisch niedrig (1. Quintil), moderat (2. – 4. Quintil) und hoch

(5. Quintil) klassifiziert. Die PAC oder der ARR wurden in den Modellen entweder als kontinuierliche oder als kategoriale (alters- und geschlechtsspezifische Tertile) Variablen berücksichtigt. Odds ratios und 95% Konfidenzintervalle wurden berechnet. Die dem Modell zugrundeliegende Annahme der proportionalen Odds wurde mit einem Chi-Quadrat Score Test[102] bestätigt. Im quantilen Regressionsmodell wurden abhängige sowie unabhängige Variablen kontinuierlich verwendet. Da dieses Regressionsmodell eine Normalverteilung der Daten nicht voraussetzt, war eine Transformation der abhängigen Variablen nicht notwendig. Es wurden Regressionskoeffizienten für den Median mit 95% Konfidenzintervallen berechnet.

Zur Beurteilung des Zusammenhangs zwischen der PAC und dem MetS oder seinen Einzelkomponenten wurden logistische Regressionsmodelle[101,102] konstruiert. Das MetS und seine Einzelkomponenten sind dichotome Variablen. Die PAC wurde in alters- und geschlechtsspezifische Quintile kategorisiert. Odds ratios und 95% Konfidenzintervalle wurden berechnet. Alle Analysen wurden separat für die SHIP-1 und die KORA F4 Studienpopulation durchgeführt, da signifikante Interaktionen (p-Wert <0,10) zwischen Studienregion und der PAC nachweisbar waren. Die Daten wurden für ein Ausscheiden zwischen Basis- und erster Follow-up Untersuchung basierend auf soziodemographischen und gesundheitsbezogenen Variablen gewichtet.

Die Auswahl der Kovariaten für die logistischen, ordinalen und quantilen Regressionsmodelle erfolgte hypothesenbasiert auf Grundlage früherer Studien zum Zusammenhang zwischen der PAC und dem MetS bzw. der FMD. Die Selektion der Kovariaten wurde mit klinisch tätigen Medizinern abgestimmt. Alle statistischen Analysen erfolgten mit SAS 9.1 (SAS Institute Inc., Cary, NC, USA) oder SPSS 17.0 (SPSS GmbH Software, Munich, Germany).

3. ERGEBNISSE

3.1. Referenzwerte

Die Referenzpopulation bestand aus 1347 Probanden, darunter 416 jüngere Männer und 618 jüngere Frauen zwischen 25-54 Jahren sowie 130 ältere Männer und 183 ältere Frauen zwischen 55-74 Jahren. Ein Einfluss der Blutabnahmezeit und des Nüchternstatus auf die PAC, PRC und den ARR wurde nicht festgestellt.

Die mittlere PAC und PRC war bei den Männern in allen Altersstufen signifikant höher als bei den Frauen. Der ARR war bei den jüngeren Frauen signifikant höher als bei den jüngeren Männern. Der ermittelte Referenzbereich für die PAC ist niedriger und schmaler bei älteren im Vergleich zu jüngeren Männern und Frauen. Das gleiche gilt für den PRC Referenzbereich der Männer. Der PRC Referenzbereich der Frauen sowie der ARR Referenzbereich der Männer und der Frauen sind bei den Älteren breiter als bei den Jüngeren (Tabelle 2).

Tabelle 3. Median und Referenzbereiche für die PAC, PRC, und den ARR

	Altersgruppen [Jahre]	**Median**		**Referenzbereich**	
		Männer	Frauen	Männer	Frauen
PAC [ng/l]	25-54	48,0	38,0	12,0-140,0	5,0-139,0
	55-74	43,0	34,0	6,0-113,0	5,0-130,0
	Alle Altersstufen	47,0	38,0	12,0-140,0	5,0-134,0
PRC [ng/l]	25-54	10,9	7,2	3,5-26,9	2,6-21,1
	55-74	8,2	6,6	2,3-22,7	1,7-23,5
	Alle Altersstufen	10,4	7,1	3,2-26,9	2,4-21,1
ARR	25-54	4,7	5,2	1,4-14,2	0,9-20,3
	55-74	5,4	5,1	0,9-22,4	0,7-25,5
	Alle Altersstufen	4,9	5,1	1,4-16,2	0,9-20,9

PAC, Plasma Aldosteron Konzentration; PRC, Plasma Renin Konzentration; ARR, Aldosteron-Renin-Quotient.
Alle Daten wurden für eine Nicht-Teilnahme an der Basisuntersuchung sowie für ein Ausscheiden zwischen Basis- und erster Follow-up Untersuchung gewichtet.

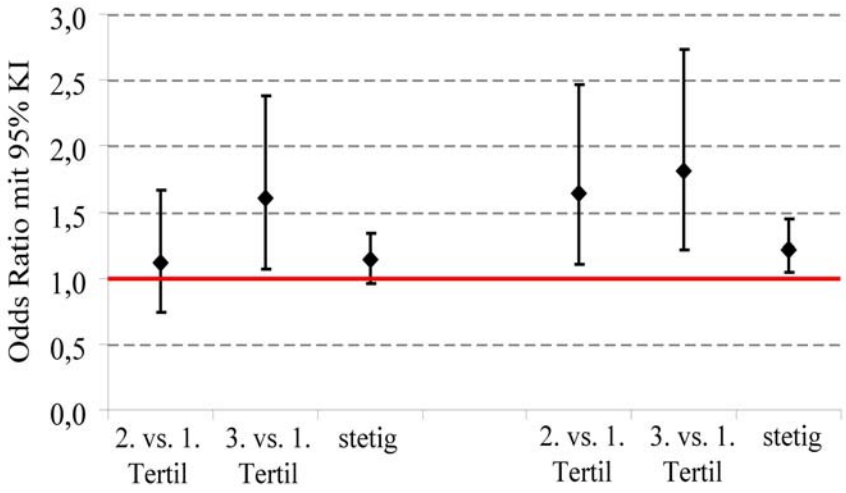
Jüngere Frauen im Alter von 25-54 Jahren, die Östrogene in Form von oralen Kontrazeptiva einnahmen, hatten im Mittel eine niedrigere PRC und einen höheren ARR als jüngere Frauen, die keine Östrogene einnahmen. Bei älteren Frauen zwischen 55-74 Jahren wurden keine signifikanten Unterschiede in der PRC oder dem ARR in

Abhängigkeit von einer menopausalen Hormonersatztherapie festgestellt. Für 25-54-jährige Frauen wurden daher separate Referenzwerte mit bzw. ohne Einnahme von Östrogenen berechnet (PRC: 2,4-17,5 ng/l und 2,8-21,2 ng/l für Frauen mit und ohne Östrogeneinnahme, ARR: 1,1-24,1 und 0,8-17,9 für Frauen mit und ohne Östrogeneinnahme).

3.2. FMD

Der Zusammenhang zwischen der PAC bzw. dem ARR und der FMD in der SHIP-1 Studie wurde anhand der Daten von 972 Probanden im Alter zwischen 25 und 88 Jahren geprüft. Es zeigte sich, dass bei 25-49-jährigen Probanden mit einer PAC bzw. einem ARR im obersten Tertil der Median der FMD Werte signifikant niedriger war als bei Probanden mit einer PAC bzw. einem ARR im untersten Tertil. In den voll adjustierten ordinalen Regressionsmodellen konnten Assoziationen zwischen hoher PAC bzw. hohem und moderatem ARR und verminderter FMD bestätigt werden. Zusätzlich wurde beobachtet, dass pro Anstieg des ARR um eine Standardabweichung die Chance für eine verminderte FMD um den Faktor 1,22 erhöht ist. Bei Probanden älter als 49 Jahre, wurden keine Assoziationen zwischen der PAC oder dem ARR und der FMD beobachtet (Abbildung 3).

Abbildung 3. Odds ratios mit 95% Konfidenzintervallen (KI) basierend auf ordinaler Regression für niedrige FMD in 25-49-jährigen Probanden.



FMD, Flussvermittelte Vasodilatation. FMD wurde in niedrig (1. Quintil), moderat (2.-4. Quintil), und erhöht (5. Quintil) kategorisiert. PAC und ARR gingen als stetige oder kategorisierte (alters- und geschlechts-spezifische Tertile) Variablen ins Modell ein. Alle Modelle wurden für Geschlecht, Alter, systolischen und diastolischen Blutdruck, Taillenumfang, Rauchstatus, Diabetes Mellitus, Sport und den Zeitraum zwischen Blutabnahme und FMD Messung adjustiert.

Bei der Analyse von 871 Probanden mit einer PAC und einem ARR innerhalb des Referenzbereichs[106] zeigten sich ähnliche Ergebnisse. Auch bei hoch-normaler PAC oder hoch-normalem ARR konnte eine Assoziation mit einer verminderten FMD nachgewiesen werden. Eine signifikante Assoziation wurde ebenfalls detektiert, wenn der ARR kontinuierlich ins Modell einging. Alle beschriebenen Assoziationen wurden wiederum nur bei 25-49-jährigen Probanden aber nicht bei älteren Probanden nachgewiesen. Eine zusätzliche Adjustierung der Modelle für den Blutabnahmezeitpunkt produzierte keine abweichenden Ergebnisse.

Im voll adjustierten quantilen Regressionsmodell wurde die Assoziation zwischen dem ARR und der FMD in jüngeren Probanden weiter bestätigt. Der Anstieg des ARR um eine Einheit resultierte in einer Verminderung der FMD um -0,09% (95%KI -0,16%; -0,02%). Bei Probanden die 50 Jahre oder älter waren, wurden keine Zusammenhänge zwischen der PAC und der FMD gefunden.

3.3. MetS

Der Zusammenhang zwischen der PAC und dem MetS wurde in SHIP-1 anhand der Daten von 2830 Probanden und in KORA F4 anhand der Daten von 2901 Probanden geprüft. In beiden Studien war eine hohe Prävalenz des MetS zu verzeichnen: 48,1% der Männer und 34,8% der Frauen in SHIP-1 sowie 42,7% der Männer und 27,5% der Frauen in KORA F4 waren betroffen. Auch bei den 1713 SHIP-1 und 2033 KORA F4 Probanden, die keine PAC verändernde Medikation einnahmen, konnte diese hohe Prävalenz bestätig werden: 37,0% der Männer und 19,2% der Frauen in SHIP-1 sowie 29,3% der Männer und 14,9% der Frauen in KORA F4 waren betroffen.

Sowohl in der SHIP-1 als auch in der KORA F4 Studienpopulation zeigten sich bei Einschluss als auch bei Ausschluss von Probanden mit Einnahme von PAC verändernder Medikation Assoziationen zwischen der PAC und dem MetS sowie erhöhten Triglyceriden (Tabelle 4). Probanden mit einer PAC im obersten Quintil hatten im Vergleich zu Probanden mit niedrigerer PAC signifikant erhöhte Chancen für ein MetS oder erhöhte Triglyceride. In SHIP-1 wurden zusätzlich Assoziationen zwischen der PAC und erhöhtem Taillenumfang sowie zwischen der PAC und erniedrigtem HDL-Cholesterol beobachtet. In der Hauptanalyse zeigte sich keine Assoziation zwischen der PAC und erhöhtem Blutdruck. In einer Sensitivitätsanalyse mit einer modifizierten Definition des erhöhten

Blutdrucks (systolischer oder diastolischer Blutdruck ≥140 oder ≥90 mmHg) wurde eine signifikante Assoziation in SHIP-1 entdeckt.

Tabelle 4. Assoziation zwischen der Plasma Aldosteron Konzentration (PAC) und dem Metabolischen Syndrom (MetS) und seinen Komponenten. Odds ratios (OR) und 95% Konfidenzintervalle (KI) für das 5. vs. 1.-4. PAC Quintil sind dargestellt.

	SHIP-1		**KORA F4**	
	Alle Probanden	**Probanden ohne Medikation***	**Alle Probanden**	**Probanden ohne Medikation***
N	2830	1713	2901	2033
OR (95%-KI) für MetS	1,64 (1,38-1,95)	1,51 (1,19-1,91)	1,38 (1,15-1,64)	1,28 (1,01-1,62)
OR (95%-KI) für Komponenten des MetS				
Erhöhter Taillenumfang	1,71 (1,41-2,09)	1,54 (1,23-1,93)	1,07 (0,89-1,29)	0,99 (0,81-1,21)
Erhöhte Glukose	1,49 (1,14-1,95)	1,24 (0,67-2,30)	1,10 (0,92-1,33)	0,95 (0,75-1,22)
Niedriges HDL-Cholesterol	1,34 (1,13-1,58)	1,36 (1,10-1,69)	1,08 (0,89-1,31)	1,29 (1,01-1,65)
Erhöhte Triglyceride	1,47 (1,22-1,77)	1,42 (1,09-1,84)	1,27 (1,06-1,52)	1,35 (1,08-1,70)
Erhöhter Blutdruck	1,11(0,93-1,34)	1,15 (0,96-1,38)	1,09 (0,87-1,37)	1,00 (0,80-1,26)

HDL-Cholesterol, High density lipoprotein Cholesterol; KORA, Kooperative Gesundheitsforschung in der Region Augsburg; SHIP, Study of Health in Pomerania

Alle logistischen Regressionsmodelle wurden adjustiert für Alter, Geschlecht, Kaliumkonzentration und Einnahme von Östrogenen bei Frauen. *Ausschluss von Probanden die PAC erhöhende (Diuretika) oder erniedrigende Medikation (Betablocker und weitere Antiadrenergika, Kalziumkanalblocker, ACE Hemmer, Angiotensin II Rezeptor Antagonisten) einnahmen. Modelle die alle Probanden einschließen wurden zusätzlich für Einnahme von PAC erhöhender oder erniedrigender Medikation adjustiert. Das gilt nicht für Modelle mit erhöhtem Blutdruck oder MetS als abhängiger Variable.

4. DISKUSSION

4.1. Referenzwerte

Die vorgestellten Referenzbereiche für die PAC, PRC und den ARR sollen Mediziner bei der Diagnose und Verlaufskontrolle von Erkrankungen des RAAS unterstützen. Wie erwartet, beobachteten wir kleine Variationen in den Referenzwerten in Abhängigkeit vom Alter und Geschlecht. Nach Veröffentlichung dieser Studienergebnisse publizierten Kerstens und Kollegen[38] eine vergleichbare Studie. Sie untersuchten 100 gesunde, normotensive Männer und Frauen zwischen 20 und 70 Jahren unter regulärer Diät und nach einem dreitägigen Salzbelastungstest. Referenzwerte für die Konzentration der SAC, PRC und des ARR wurden als zentrales 95% Intervall der jeweiligen Verteilung vor und nach der Salzbelastung bestimmt. Die obere Referenzgrenze für die SAC, PRC und den ARR bei Probanden unter regulärer Diät ergab bei Kerstens und Kollegen[38] höhere obere Referenzgrenzen als in SHIP-1. Besonders deutlich unterscheiden sich die oberen Referenzgrenzen für die SAC bei Kerstens und Kollegen (Männer und Frauen: 827 pmol/l, entspricht 298 ng/l) und die PAC in SHIP-1 (Männer: 140 ng/l, Frauen: 134 ng/l). Die oberen Referenzgrenzen für die PRC und den ARR liegen dichter beieinander. (Kerstens und Kollegen. PRC 29,6 ng/l; ARR 81,3 pmol/ng; entspricht einem ARR von 29,3 in SHIP-1; SHIP-1 PRC Männer 26,9 ng/l, Frauen 21,1 ng/l, ARR Männer 16,2, Frauen 20,9). Diese Unterschiede können durch eine Vielzahl an Faktoren verursacht sein, z. B. durch Unterschiede bei der Blutabnahme,[28,107] dem Salzkonsum,[36] der Analytik[31,32] oder durch die Größe der Studienpopulation. So war die Positionierung der Probanden bei der Blutabnahme unterschiedlich (SHIP-1: liegend, Kerstens und Kollegen: sitzend), der Salzkonsum der SHIP-1 Probanden unbekannt und während die gleichen Labormethoden verwendet wurden, ist in SHIP-1 die PAC im EDTA-Plasma, bei Kerstens und Kollegen jedoch die SAC gemessen worden. Weiterhin bestand die Referenzpopulation von Kerstens und Kollegen[38] aus 100 gesunden Probanden, die SHIP-1 Referenzpopulation hingegen aus 1347 gesunden Probanden, wobei die größere Fallzahl robustere Ergebnisse liefert.

Neben Referenzwerten basierend auf einer gesunden Population, haben eine Reihe von Studien[35,108-112] Grenzwerte für einen erhöhten ARR im Rahmen des PAL Screenings ermittelt. Im Großteil dieser Studien[35,108,111,112] wurden Grenzwerte für den ARR ermittelt, indem Individuen mit PAL solchen ohne PAL gegenübergestellt wurden und der Punkt

bestimmt wurde, an dem die beste Trennung beider Gruppen erzielt wird. Während diese Studie Patienten aus der hausärztlichen Versorgung[110,112] oder bestimmten Hypertoniezentren[35,108,109,111] einschließen, stammen die SHIP-1 Referenzwerte aus der gesunden Allgemeinbevölkerung. Ein ARR oberhalb des SHIP-1 Referenzbereichs ist daher nicht gleichbedeutend mit dem Vorliegen eines PAL. Gleichzeitig ist bei einem ARR innerhalb des SHIP-1 Referenzbereichs das Vorliegens eines PAL unwahrscheinlich.

Die wesentliche Stärke der vorgestellten Studie liegt in der umfassenden Phänotypisierung der Studienprobanden, die es ermöglichte eine gesunde Referenzpopulation zu selektieren. So wurden bei jedem Probanden drei Blutdruckmessungen im Abstand von drei Minuten durchgeführt. Der Mittelwert aus zweiter und dritter Blutdruckmessung wurde für die statistische Analyse verwendet, um einen *white-coat hypertension* Effekt[113] zu vermeiden. Weiterhin zeichnet sich die Referenzpopulation durch ihre große Fallzahl aus, die eine sichere Abschätzung des 95% Referenzbereichs zulässt. Leider konnte die Bestimmung der Referenzwerte nicht anhand der Daten der SHIP-0 Basisuntersuchung erfolgen, da nicht alle präanalytischen Vorgaben für die Messung der PAC und PRC erfüllt waren. Die ermittelten SHIP-1 Referenzwerte sind dennoch repräsentativ für die Bevölkerung der Studienregion, da die Analysen entsprechend gewichtet wurden. Eine wesentliche Limitation der Studie ist die Einschränkung der Übertragbarkeit der Studienergebnisse auf andere Labormethoden, z. B. andere PAC oder PRC Assays. Eine weitere Limitation der Studie stellt die einmalige Blutabnahme dar. Es ist strittig, ob eine einmalige Messung das Hormonprofil eines Probanden exakt wiedergeben kann.[33] Des Weiteren können keine Angaben zum Salzkonsum und seinem Einfluss auf die Referenzwerte für PAC, PRC und ARR getroffen werden, da entsprechende Angaben in SHIP-1 nicht erhoben wurden.

4.2. FMD

In der vorliegenden Arbeit wurde ein inverser Zusammenhang zwischen dem ARR und der FMD bei Probanden zwischen 25 und 49 Jahren beobachtet.[114] In der gleichen Altersgruppe wiesen Individuen mit hoher sowie hoch-normaler PAC gegenüber Individuen mit niedriger sowie niedrig-normaler PAC eine höhere Wahrscheinlichkeit für eine verminderte FMD auf. Damit ergänzt und erweitert die vorliegende Studie die bisher bekannten Forschungsergebnisse. In früheren Studien mit PAL Patienten[63,64] wurden schädigende Effekte einer hohen PAC und eines hohen ARR auf das Endothel

nachgewiesen. So war die FMD bei hypertensiven Patienten mit PAL niedriger als bei hypertensiven Patienten ohne PAL.[63,64] Weiterhin wurden Verbesserungen in der FMD nach erfolgreicher medikamentöser oder operativer Therapie des PAL berichtet.[63,64] Auch bei Probanden ohne PAL wurden bereits Assoziationen zwischen einzelnen Komponenten des RAAS und der Endothelfunktion nachgewiesen.[65,115] In der Framingham Heart Study[65] wurde bei hyper- und normotensiven Probanden ein Zusammenhang zwischen Renin im Plasma und der FMD detektiert. Ein Zusammenhang zwischen der SAC und der FMD wurde nicht gefunden.[65] Diese Analyse ist allerdings durch den großen Abstand (im Mittel 2,9 Jahre) zwischen Blutabnahme mit Biomarkerbestimmung und FMD Messung limitiert.

Bei der Analyse der SHIP-1 Daten wurde kein Zusammenhang zwischen der PAC oder dem ARR und der FMD bei Probanden älter als 49 Jahre ermittelt. Ein steigendes Alter kann unabhängig von atherosklerotischen Erkrankungen zu einer Verringerung der vaskulären Funktion beitragen.[116,117] Gleichzeitig bewirken im Laufe des Lebens angesammelte kardiovaskuläre Risikofaktoren funktionelle und strukturelle Veränderungen am vaskulären Endothel.[57,116,118] Daher ist es denkbar, dass bei älteren Individuen mit bereits eingeschränkter Endothelfunktion eine hohe PAC bzw. ein hoher ARR nicht die gleichen Effekte ausüben wie bei jungen Probanden mit normaler Endothelfunktion.

Die vorgestellte Studie zeichnet sich wiederum durch die große, gut charakterisierte Studienpopulation aus. Alle Untersuchungen wurden von geschultem Personal nach standardisierten Richtlinien durchgeführt. Zur Qualitätssicherung der FMD Messung wurden alle Untersucher vor und während der Datenerhebung regelmäßig zertifiziert[97]. Andererseits müssen vier wichtige Limitationen herausgestellt werden: Erstens ist die Subpopulation von Probanden die an der FMD Messung teilnahmen nicht repräsentativ für die gesamte SHIP-1 Studienpopulation. Die FMD Teilnehmer waren im Schnitt jünger und möglicherweise gesünder als die Nicht-Teilnehmer. Zweitens war bei ca. 20% der Probanden der Zeitraum zwischen Blutabnahme und FMD Messung länger als 90 Tage. Da sich in einem solchen Zeitraum sowohl die PAC, der ARR als auch die FMD verändern können, führt dies möglicherweise zu einer Verzerrung in der Analyse. Drittens wurde in SHIP-1 ganztätig Blut abgenommen. Da es keinen Unterschied in der PAC, PRC, und dem ARR nach der Blutabnahmezeit gab[106] und die Ergebnisse der Regressionsmodelle durch zusätzliche Adjustierung für den Blutabnahmezeitpunkt nicht verändert wurden, liegt aber vermutlich keine Beeinflussung der Ergebnisse vor. Viertens kann die vorgestellte epidemiologische Querschnittsstudie keine kausalen Erklärungen für die beobachteten

Zusammenhänge zwischen der PAC oder dem ARR mit der FMD liefern, sondern nur der Hypothesengenerierung dienen.

4.3. MetS

Sowohl in SHIP-1 als auch in KORA F4 wurden Zusammenhänge zwischen der PAC und dem MetS sowie Fettstoffwechselstörungen detektiert.[119] Eine niedrigere PAC war mit einem erhöhten Odds Ratio für ein MetS, niedriges HDL-Cholesterol oder erhöhte Triglyceride assoziiert. In früheren epidemiologischen Quer- und Längsschnittsstudien wurden Assoziationen zwischen der PAC bzw. der SAC und dem MetS[83,84] bzw. inzidentem MetS[120] beobachtet. Auch Assoziationen zwischen der PAC und den Fettstoffwechselkomponenten des MetS; erhöhter Taillenumfang,[84,91] niedriges HDL-Cholesterol[84] und erhöhte Triglyceride,[91] wurden bereits nachgewiesen. Zusätzlich ist bekannt, dass die Prävalenz des MetS bei Patienten mit PAL höher ist als bei Patienten mit essentieller Hypertonie.[83] In keiner dieser Analysen wurde die Fallzahl aus SHIP-1 und KORA F4 erreicht.

Da der Zusammenhang zwischen erhöhter SAC und inzidenter Hypertonie als gesichert gilt,[55] erschien es zunächst überraschend, dass weder in SHIP-1 noch in KORA F4 eine signifikante Assoziation zwischen der PAC und erhöhtem Blutdruck festgestellt wurde. Allerdings wurden alle Studienteilnehmer unter regulärer Medikation untersucht und ein hoher Anteil (SHIP-1: 39,5%, KORA F4: 29,9%) nahm antihypertensive Präparate ein, die Veränderungen der PAC bewirken. Nach Ausschluss dieser Probanden, reduzierte sich der Anteil der Individuen mit erhöhtem Blutdruck um 55,5% sowie der Anteil der Probanden mit MetS um 58,1%. Die Analysen in der resultierenden kleineren und selektierten Studienpopulation hatten damit eine geringere statistische Power. Assoziationen zwischen der PAC und erhöhtem Blutdruck wurden auch in dieser Population nicht beobachtet. Nach Anhebung der Grenzwerte für einen erhöhten systolischen und diastolischen Blutdruck von 130 und 85 mmHg auf 140 und 90 mmHg wurde jedoch in SHIP-1 eine signifikante Assoziation entdeckt. Ein Zusammenhang zwischen der PAC und dem Schweregrad der Hypertonie ist daher denkbar.

Die Mechanismen, die dem Zusammenhang zwischen der PAC und den Komponenten des MetS zugrunde liegen, sind bisher nur teilweise geklärt. Diskutiert werden Hypothesen, nach denen eine erhöhte PAC Inflammationsprozesse und einen Anstieg des oxidativen Stress verursachen,[71] die wiederum die Entwicklung von

Insulinresistenz und verminderter ß-Zellfunktion,[71,86-88] endothelialer Dysfunktion und Hypertonie[71] unterstützen können. Laut einer weiteren Hypothese[72,121,122] können in einem sich selbst verstärkenden Prozess humane Adipozyten eine Stimulation der Aldosteronsynthese bewirken. Das Aldosteron fördert dann wiederum die Adipogenese.

Ebenso wie bei den vorangegangenen Analysen ist die große Datenbasis und die standardisierte Datenerhebung eine Stärke der vorliegenden Studie. Das Auffinden ähnlicher Ergebnisse in den Stichproben aus Nordost- und Süddeutschland bestätigt die gefundenen Zusammenhänge. Limitationen der Analyse ergeben sich aus den unterschiedlichen Blutabnahmebedingungen und Methoden zur Bestimmung der PAC, Glukose, Triglyceride und dem HDL-Cholesterol. Daher war es notwendig studienspezifische Grenzwerte für erhöhte Glukose und Triglyceride sowie für die PAC Quintile festzulegen. Weiterhin waren alle Probanden in SHIP-1 und KORA F4 unter regulärer Diät mit unbekanntem Salzkonsum und ein Teil der Probanden unter antihypertensiver Medikation. Beide Faktoren wirken auf die PAC[22,36] und können zu einer Verzerrung der Messergebnisse geführt haben. Bei der vorliegenden Studie treffen zwei weitere Limitationen zu, die bereits bei den anderen vorgestellten Analysen erwähnt wurden. Erstens beruhen alle Laboranalysen auf Einzelmessungen. Zweitens erlaubt auch das Design dieser Analyse nur die Hypothesengenerierung, aber nicht die Ableitung kausaler Zusammenhänge.

5. ZUSAMMENFASSUNG UND AUSBLICK

Die in dieser Arbeit vorgestellten Ergebnisse bieten einen Einblick in die Wirkung ausgewählter Komponenten des RAAS auf das kardiovaskuläre System und den Metabolismus. Erstmalig in der Literatur wurden Referenzwerte für die PAC, PRC und den ARR aus einer großen populationsbasierten Studie ermittelt. Diese können im Rahmen der Diagnostik und Verlaufskontrolle bei Erkrankungen des RAAS angewandt werden. Darüber hinaus können sie auch bei wissenschaftlichen Analysen eingesetzt werden, da sie eine Klassifizierung von Studienteilnehmern anhand der PAC, PRC oder des ARR zulassen. Anhand der analysierten Daten konnte weiterhin gezeigt werden, dass eine hohe PAC oder ein hoher ARR zu einer Verminderung der FMD bei Männern und Frauen unter 50 Jahren beitragen und dementsprechend die Progression von subklinischer Atherosklerose vorantreiben können. Diese Analyse trug zur Erweiterung des Wissenstands bei, da zum Zeitpunkt der Veröffentlichung keine populationsbasierte Studie vorlag, die die Assoziationen zwischen der PAC oder dem ARR auch innerhalb des Referenzbereichs mit der FMD untersuchte. Des Weiteren wurde ein Zusammenhang zwischen hoher PAC und dem vermehrten Auftreten des MetS als auch Störungen des Fettstoffwechsels aufgezeigt. Diese Zusammenhänge wurden in zwei großen deutschen Studien beobachtet. Ihre Ergebnisse bestärken sich somit gegenseitig. Zusammenfassend bekräftigen die durchgeführten Analysen die Hypothese, dass Störungen des RAAS mit pathophysiologischen kardiovaskulären und metabolischen Veränderungen einhergehen.

In nachfolgenden Analysen wäre es von großem Interesse die beobachteten querschnittlichen Assoziationen im Längsschnitt zu betrachten. Dies würde es ermöglichen den prädiktiven Wert der PAC, PRC und des ARR hinsichtlich der FMD und des MetS zu beurteilen. Nach Abschluss der Datenerhebung in SHIP-2 sind solche Analysen angestrebt. Gleichermaßen wäre es von großem Interesse Analysen auf dem Gebiet der „-omics“ Forschung, beispielsweise der Genomics, Transcriptomics und der Metabolomics durchzuführen. Anhand solcher Analysen können personalisierte Risikoprofile erstellt und Signalwege des Renins und Aldosterons identifiziert werden. So können sie zur Aufklärung der Pathogenese von kardiovaskulären und metabolischen Erkrankungen im Zusammenhang mit dem RAAS beitragen.

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7. ANHANG

7.1. Wissenschaftliche Publikationen

7.1.1. Reference intervals for aldosterone, renin, and the aldosterone-to-renin ratio in the population-based Study of Health in Pomerania (SHIP-1).

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Reference Intervals for Aldosterone, Renin, and the Aldosterone-to-Renin Ratio in the Population-based Study of Health in Pomerania (SHIP-1)

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Key words
- primary aldosteronism
- active renin concentration
- plasma aldosterone concentration
- endocrine hypertension

Abstract

The renin-angiotensin-aldosterone system plays a key role in the regulation of human blood pressure. The aldosterone-to-renin ratio (ARR) is widely accepted for screening the primary hyperaldosteronism (PAL). Various cutoffs for positive PAL screening have been defined in patient cohorts from endocrinological referral centers and primary care. However, the distribution of the ARR in the general population is largely unknown. We aim to provide reference ranges for plasma aldosterone concentration (PAC), plasma renin concentration (PRC), and the ARR for the general population of north-east Germany. A cohort of 3 300 subjects participated in the first follow-up of the longitudinal, population-based Study of Health in Pomerania (SHIP). PAC and PRC were measured by radioimmunometric procedures. The reference interval was defined as the central 95% range between the 2.5th and 97.5th percentiles. A reference population comprising 1 347 healthy subjects was selected. Sex and age-specific (25–54 and 55–74 years) reference ranges are presented. The upper reference limit for the ARR was 14.2 and 20.3 in younger, and 22.4 and 25.5 in older men and women, respectively. Time of blood sampling had no influence on the ARR, while beta blockers, and agents acting on the renin-angiotensin system were associated with higher and lower ARR, respectively. Our upper reference limit for the ARR is clearly lower than previously reported values from studies of hypertensive patients in primary care or hypertension referral centers. We confirm that PAC and PRC are associated with various factors, including sex, age, intake of estrogen, and various antihypertensive medications.

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Introduction

The renin-angiotensin-aldosterone system plays a key role in regulating human blood pressure and sodium balance [1–3]. Insufficient aldosterone synthesis may lead to hypotension and hyperkalemia, hyperchloremic acidosis, and impaired renal sodium conservation [4,5]. On the contrary, excessive aldosterone levels, as referred to as hyperaldosteronism, contribute to vascular dysfunction, renal injury, cardiovascular mortality [6,7], obesity, and the metabolic syndrome [8]. Two types of hyperaldosteronism, primary and secondary hyperaldosteronism, are differentiated. In the latter, high plasma renin activity causes hypersecretion of aldosterone [2]. In primary hyperaldosteronism (PAL), aldosterone is produced independently from the RAAS. In most cases this is either due to an aldosterone-producing adenoma, or uni- or bilateral adrenal hyperplasia [9]. PAL is considered a common cause of hypertension [10,11]. For hypertensive patients, prevalences of PAL between 5–12% were reported [12–14]. In PAL, a normokalemic and a less frequent hypokalemic variant are differentiated – both show a high prevalence of comorbidities [15]. Compared to patients with essential hypertension, patients with PAL exhibit a higher risk of cardiovascular events, including myocardial infarction, stroke, and atrial fibrillation [16].

For the clinical assessment of hypertensive patients measurements of aldosterone and renin are fundamental. In screening for PAL, the aldosterone-to-renin ratio (ARR) is widely applied, as its sensitivity is superior to measurements of aldosterone or renin alone [17]. Due to its limited specificity the ARR is not diagnostic for PAL, and confirmatory testing (e.g., saline infusion test, fludrocortisone suppression test) is mandatory

[18, 19]. In patients from hypertension referral centers and primary care, various cutoff values for PAL screening have been proposed. They vary strongly due to the absence of standardized test conditions and small reference populations [20]. At the same time, the ARR distribution in the general population is largely unknown.

Our aim was to provide reference ranges for plasma aldosterone concentration (PAC), plasma renin concentration (PRC), and the ARR in a large, population-based study of the general population of north-east Germany. We expected, as previously described, to find relevant sex differences in PAC, PRC, and the ARR and a modest decrease with age [21]. Moreover, in sensitivity analyses we aimed to assess the influence of medication intake, fasting status, and time of blood sampling on all three parameters.

Subjects and Methods

Study population

The Study of Health in Pomerania (SHIP) is a longitudinal, population-based study, conducted in West Pomerania, in the north-east of Germany. For the baseline examination, SHIP-0, a representative sample from the entire population of 158 864 adults aged 20–79 years living in the area was drawn. By a two-stage cluster sampling method, adopted from the WHO MONICA Project Augsburg, Germany, 7008 men and women were selected. A total of 4310 subjects participated (68.8% of eligible subjects) in the examinations between October 1997 and May 2001 [22].

The first follow-up examination, designated as SHIP-1, was conducted five years later. All baseline participants were re-invited. Among those, 361 subjects were lost to follow-up due to death (n = 231) or migration (n = 130). Of the remaining 3949 eligible subjects, 3300 participated in the follow-up examination between March 2003 and July 2006 (83.6% of eligible subjects). All participants gave written informed consent. The study conformed to the principles of the Declaration of Helsinki as reflected by an a priori approval of the Ethics Committee of the Board of Physicians Mecklenburg-West Pomerania at the University of Greifswald.

Data collection

In SHIP-0 and SHIP-1, information on medical history as well as behavioral, and socio-demographic characteristics were obtained by a computer-aided personal interview. Additionally, participants were asked to bring all medication taken in the last seven days before the examination. The drugs were categorized according to the anatomical-therapeutic-chemical (ATC) classification code.

During the physical examination, systolic and diastolic blood pressures were measured after a rest period of at least five minutes on the right arm of the seated participant. In intervals of three minutes, three measurements were obtained, using a digital blood pressure monitor (HEM-705CP, OMRON Corporation, Tokyo, Japan). For statistical analyses the mean of the second and third measurements was used. Subjects with systolic blood pressure ≥ 140 mmHg or diastolic blood pressure ≥ 90 mmHg or self-reported intake of antihypertensive medication were considered hypertensive.

Laboratory measurements

After a rest period of ten minutes, blood was taken from the cubital vein of subjects in the supine position by certified examiners. The sampling took place between 8.00 AM and 8.00 PM, with the majority (86%) of samples taken between 9.00 AM and 3.00 PM. Subjects were on their regular medication and decided themselves to undergo examinations either fasting (10%) or nonfasting (90%). Serum and plasma were stored at −80 °C. PAC and PRC were measured in EDTA plasma by radioimmunometric procedures (PAC, Coat-A-Count Aldosterone, Siemens Healthcare Diagnostics, Eschborn, Germany; PRC, Renin III generation, Cisbio Bioassay, Bagnols-sur-Cèze Cedex, France).

The analytical sensitivity of the PAC assay was 11 ng/l and its measurement range was between 25–1200 ng/l. However, values between 5–25 ng/l (n = 686) were determined with adequate reliability and were also used for the present analyses. All values below 5 ng/l (n = 80) were set to 5 ng/l. Inter- and intra-assay coefficients of variation were 15.7 and 5.4% in low and 3.8 and 2.3% in high concentrations, respectively. According to the manufacturer, results in serum and heparinized plasma are comparable, while PAC is on average 15% higher in EDTA plasma. Values above 1200 ng/l (n = 1) were measured in dilution.

The analytical sensitivity of the PRC assay was 1 ng/l, its measurement range was between 1 and 320 ng/l. Values between 0.4 and 1.0 ng/l were determined in ten subjects and used for the analysis. All values below 0.4 ng/l (n = 5) were set to 0.4 ng/l. Values above 320 ng/l in PRC (n = 15) were measured in dilution. Inter- and intra-assay coefficients of variation were 5.0 and 3.6% in low and 4.0 and 0.9% in high concentrations, respectively. The standards in the PRC kits were calibrated against the international reference preparation (WHO 68/356).

Potassium was measured in serum by indirect potentiometry with ion-selective electrodes (Dimension RxL Max HM, Siemens Healthcare Diagnostics, Eschborn, Germany). Hypokalemia was defined as serum potassium concentrations below 3.5 mmol/l. Serum creatinine levels were determined with the Jaffé method (Hitachi 717, Roche Diagnostics GmbH, Mannheim, Germany). The creatinine clearance (CrCl) was calculated using the Cockroft-Gault formula. Renal insufficiency was defined as CrCl < 50 ml/min.

Statistical analyses

To provide representative estimates of PAC, PRC, and the ARR for the population of north-east Germany all data were weighted. The weights accounted for nonresponse to baseline (SHIP-0) and drop-out to follow-up (SHIP-1) based on socio-demographic and health-related variables.

Dichotomous variables are expressed as percent values, continuous data, except for PAC, PRC, and the ARR, are expressed as mean ± standard deviation (SD). According to Kolmogorov-Smirnov statistics, neither PAC, PRC, ARR nor their log-transformed values were normally distributed. Descriptive statistics are therefore given as median, 25th, and 75th percentiles.

The reference population was selected by excluding all participants with missing or biased PAC, PRC, and ARR levels. This comprised all subjects with suspected hyperaldosteronism (with PAC or PRC values above 1000 ng/l), hypokalemia, hypertension, pregnancy, renal insufficiency, intake of antihypertensives (ATC C02), diuretics (ATC C03), beta blockers (ATC C07), calcium channel blockers (ATC C08), and agents acting on the renin-angiotensin system (ATC C09). Due to small numbers, also subjects above 74 years of age were excluded from the reference popula-

tion. The reference interval was defined as the central 95 % range between the 2.5th and the 97.5th percentiles. The Kruskal-Wallis test was used for group comparisons. A p-value of <0.05 was considered statistically significant. Except from the Kruskal-Wallis test, and the box plots, which were created with SPSS 17.0 (SPSS GmbH Software, Munich, Germany), all statistical analyses were performed with SAS 9.1 (SAS Institute Inc., Cary, NC, USA).

Sensitivity analyses

It was previously shown that conditions of blood sampling, such as time of day, and posture of the patient [17, 23, 24] influence the ARR and/or its components. The circadian rhythms of aldosterone and renin peak in the morning and decline in the afternoon [25]. They are active in normo- and hypertensive subjects [24, 26] and might be affected by food intake [25]. We performed descriptive statistics to assess whether time of blood sampling or fasting status influence the ARR and its components in the SHIP reference population.

Levels of PAC, PRC, and the ARR are altered by various drugs [18]. While diuretics, ACE inhibitors, and calcium channel blockers are known to reduce the ARR, beta blockers increase the ratio [18]. The strength of the influence on PAL screening and whether or not subjects should withdraw or continue antihypertensive treatment before screening, are controversially discussed [18, 27–29]. We aim to compare and test differences in PAC, PRC, and the ARR between subjects under the above mentioned medication, and the reference population.

Intake of estrogen, either in oral contraceptives (ATC G03A) or hormone replacement therapy (ATC G03C, ATC G03D, ATC G03F), is frequent among female SHIP-1 participants. Although estrogen may lower PRC and subsequently increase the ARR [30, 31], we decided not to exclude these women from the reference population. Instead we determined additional reference ranges for women with and without intake of estrogen.

Results

In 36 out of 3 300 subjects, PAC and/or PRC were not measured. In two subjects extremely high PAC ($>2\,500$ and $>1\,100$ ng/l, respectively) and PRC levels ($>5\,000$ and >100 ng/l, respectively) were detected. Both were strongly suspected to have secondary hyperaldosteronism, and were excluded from the analyses. This resulted in a study population of 3 262 subjects. To form the reference population, we excluded 19 subjects with hypokalemia, 1 694 subjects with hypertension, 154 subjects with renal insufficiency, 1 327 subjects who used antihypertensives, diuretics, beta blockers, calcium channel blockers, or agents acting on the renin-angiotensin system (either alone or in combination), 12 pregnant women, and 21 subjects above 74 years (overlap between the exclusion criteria exists). This resulted in a reference population of 1 347 subjects, and a nonreference population of 1 915 subjects.

The reference population was younger, and had a lower body mass index (BMI) as well as lower systolic and diastolic blood pressures than the nonreference population (**Table 1**). While this applied for both sexes, lower potassium and PRC as well as higher ARR, were only seen in men. Differences in PAC between the reference and the nonreference population were not statistically significant. Women in the reference population had a lower BMI as well as lower systolic and diastolic blood pressures, but were slightly older than men in the reference population. Potassium, PAC, and PRC were also lower in women than in men. Yet, women had higher ARR than men (**Table 1**).

Reference ranges

In the reference population, PRC decreased significantly with age in men but not in women. Decreases with age were also seen for PAC and the ARR, however, these were not statistically significant. To allow for the decline with age in PRC, we dichotomized our reference population in younger (25–54 years) and older (55–74 years) participants. Reference intervals for PAC, PRC, and the ARR are presented for these two age groups and above all ages (**Table 2**). Younger and older women had higher upper ARR reference limits (20.3 and 25.5, respectively) than men (14.2 and 22.3, respectively), but similar PAC and PRC.

The manufacturer's reference range for PAC in EDTA plasma was 11.5–184 ng/l for both sexes and all age groups. The recommended lower reference limit was close to ours (12 and 5 ng/l for men and women, respectively), but the recommended upper reference limit was considerably higher than the one presented here (140 and 134 ng/l for men and women, respectively). For PRC the manufacturer provided age-specific reference ranges: 3.6–20.1 ng/l for 20–40 year old and 1.1–20.2 ng/l for 40–60 year old subjects. We determined broader reference intervals in the younger (25–54 years), and higher reference limits in the older (55–74 years) SHIP-1 participants.

Sensitivity analyses

Time of blood sampling was not recorded in six participants. Fasting-status was missing for two subjects. These were excluded from the analyses. Descriptive statistics revealed small fluctuations by time of blood sampling for PAC, PRC, and the ARR (**Fig. 1**). In men PAC, PRC, and the ARR declined between 9.00 AM and 2.00 PM. Afterwards, PAC and PRC increased slightly, while the ARR only increased after 4.00 PM. In women PAC, PRC, and the ARR were nearly constant over the time of day. Exceptions were seen around noon and in the late afternoon, when PAC and the ARR were slightly elevated, as well as between 2.00 and 4.00 PM, when PRC was elevated. The ARR was hardly affected by time of blood sampling. This was confirmed by group comparisons, which showed no statistically significant influence of time of blood sampling on PAC, PRC, or the ARR. Also fasting status had no statistically significant influence on the measured hormone values (**Fig. 2**).

In the nonreference population more than two thirds (68.7 %) of subjects reported the use of antihypertensives, diuretics, beta blockers, calcium channel blockers, or agents acting on the renin-angiotensin system. Among those 54.3 % received a combination, while the rest took only one of the drugs. Compared to the reference population, subjects with beta blocker intake had significantly lower PAC and PRC, but higher ARR levels (**Table 3**). The 97.5th percentile of their ARR distribution was 65.6 (over all ages and both sexes). Male subjects under treatment with agents acting on the renin-angiotensin system had lower PAC compared to the reference population, while higher PRC and lower ARR were observed in both sexes. The 97.5th percentile of the ARR distribution of subjects with intake of agents acting on the renin-angiotensin system was 14.2 (over all ages and both sexes). Use of diuretics, antihypertensives, and calcium channel blockers alone was rare, but common in combination therapy. While female subjects who received a combination of any of the above mentioned drugs had higher PAC than females from the refer-

Table 1 Characteristics of the nonreference and the reference population

Characteristics	Nonreference population		Reference population	
	Males	Females	Males	Females
	(n = 1 032)	(n = 883)	(n = 546)	(n = 801)
Age (years)	56.7 (13.5)	62.6 (13.7)*	41.7 (12.8)†	43.1 (12.0)*†
BMI (kg/m^2)	29.3 (4.1)	29.8 (5.6)	26.6 (4.1)†	25.4 (4.5)*†
Systolic BP (mmHg)	144.4 (17.7)	140.7 (19.5)*	124.5 (9.6)†	115.3 (11.2)*†
Diastolic BP (mmHg)	87.0 (11.2)	83.0 (11.4)*	79.0 (6.6)†	75.7 (6.8)*†
Hypertension	92.1 %	86.6 %	–	–
Intake‡ of				
– antihypertensives	3.2 %	2.8 %	–	–
– diuretics	12.2 %	16.6 %	–	–
– beta blockers	37.6 %	48.5 %	–	–
– calcium channel blockers	15.5 %	17.9 %	–	–
– agents acting on the renin-angiotensin system	40.3 %	44.0 %	–	–
– estrogen	–	9.5 %	–	22.8 %
Potassium (mmol/l)	4.3 (4.1–4.5)	4.3 (4.0–4.5)*	4.4 (4.2–4.6)†	4.3 (4.1–4.5)*
PAC (ng/l)	46.0 (29.0–71.0)	40.0 (24.0–61.0)*	47.0 (32.0–72.0)	38.0 (23.0–59.0)*
PRC (ng/l)	10.5 (6.0–20.1)	7.6 (4.1–13.8)*	10.4 (6.9–14.8)†	7.1 (4.7–10.6)*
ARR	4.2 (1.8–8.1)	5.0 (2.1–10.0)*	4.9 (3.1–7.1)†	5.1 (2.9–8.8)*

PAC: plasma aldosterone concentration; PRC: plasma renin concentration; ARR: aldosterone-to-renin ratio; BMI: body mass index; BP: blood pressure

Hypertension is defined as systolic and diastolic blood pressure ≥ 140/90 mmHg, and/or self reported intake of antihypertensive medication

Values are reported as mean (SD) for age, BMI, systolic and diastolic blood pressures. Because of the skewed distributions of PAC, PRC, ARR, and potassium, median values (25th and 75th percentile) are shown

* Statistically significant differences between males and females of the reference population, and males and females of the nonreference population

† Statistically significant differences between subjects of the reference and nonreference population

‡ Medication intake as mono- or combination therapy

All data were weighted according to the population of West Pomerania

Table 2 Median and reference ranges for PAC, PRC, and the ARR by sex and age groups

	Age group (years)	n		Median		Reference interval	
		Males	Females	Males	Females	Males	Females
PAC [ng/l]	25–54	416	618	48.0	38.0	12.0–140.0	5.0–139.0
	55–74	130	183	43.0	34.0	6.0–113.0	5.0–130.0
	All ages	546	801	47.0	38.0	12.0–140.0	5.0–134.0
PRC [ng/l]	25–54	416	618	10.9	7.2	3.5–26.9	2.6–21.1
	55–74	130	183	8.2	6.6	2.3–22.7	1.7–23.5
	All ages	546	801	10.4	7.1	3.2–26.9	2.4–21.1
ARR	25–54	416	618	4.7	5.2	1.4–14.2	0.9–20.3
	55–74	130	183	5.4	5.1	0.9–22.4	0.7–25.5
	All ages	546	801	4.9	5.1	1.4–16.2	0.9–20.9

PAC: plasma aldosterone concentration; PRC: plasma renin concentration; ARR: aldosterone-to-renin ratio

All data were weighted according to the population of West Pomerania

ence population, PRC was higher, and ARR was lower in those receiving a combination therapy.

Women in the reference population, who were using oral contraceptives or receiving hormone replacement therapy, exhibited lower PRC and higher ARR levels than those not taking estrogen preparations. However, these differences were statistically significant only in women up to 54 years. In women between 25–54 years the ARR reference range was 1.1–24.1 for those taking estrogens, and 0.8–17.9 for those not taking estrogens.

Discussion

To the best of our knowledge, this is the first study presenting reference values for PAC, PRC, and the ARR from a population-based study. Reference intervals for PAC, PRC, and the ARR were calculated aiming to support clinicians in diagnosis and therapeutic monitoring of endocrine forms of hypertension. As expected, the presented reference intervals varied slightly with age and sex. In concordance with the assay manufacturer we found a significant decrease in PRC with age in men, but not in women. Probably due to the higher number of females compared to males in the reference population, the reference ranges for PRC and the ARR broadened with age in women but not in men. In order to take the age-dependent variations in PRC into account, we separated our reference population into two age groups.

Analogous to the Framingham Heart Study [32], we found sex differences in PAC, PRC, and the ARR. We also found higher ARR, and lower PRC in females than in males. However, the association between sex and aldosterone was different between our study and the Framingham Study [32]. While we demonstrated lower PAC in women than in men, the Framingham Study

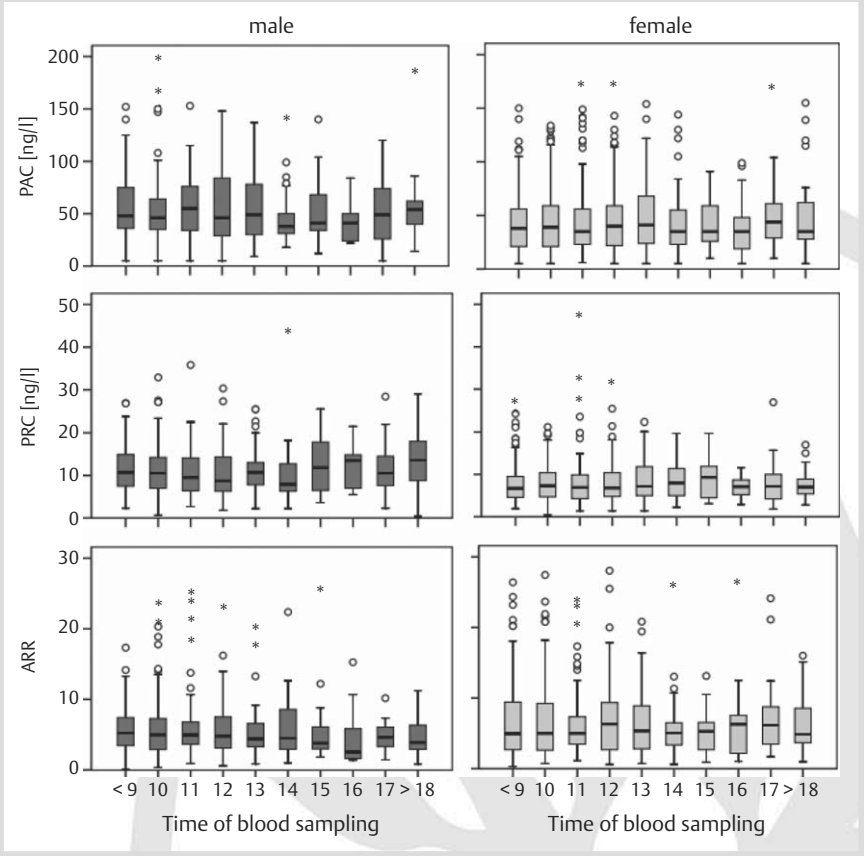

Fig. 1 Box plots for plasma aldosterone concentration (PAC) [ng/l], plasma renin concentration (PRC) [ng/l], and the aldosterone-to-renin ratio (ARR) by time of blood sampling for males and females of the reference population. Number of blood samples taken: 8.00–9.59: n = 309; 10.00–10.59: n = 270; 11.00–11.59: n = 178; 12.00–12.59: n = 179; 13.00 13.59: n = 144; 14.00–14.59: n = 81; 15.00–15.59: n = 49; 16.00–16.59: n = 35; 17.00–17.59: n = 59; after 18.00 n = 40. All data were weighted according to the population of West Pomerania.

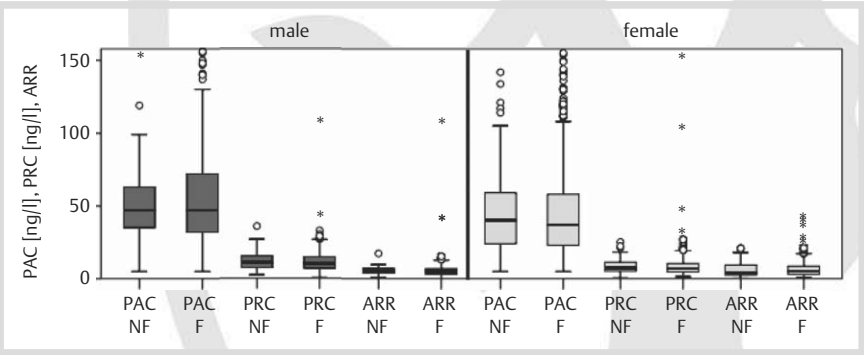

Fig. 2 Box plots for plasma aldosterone concentration (PAC) [ng/l], plasma renin concentration (PRC) [ng/l], and the aldosterone-to-renin ratio (ARR) by fasting status (NF: nonfasting, F: fasting) for males and females of the reference population. Number of fasting males: n = 56, fasting females: n = 78, nonfasting males: n = 490, nonfasting females: n = 723. All data were weighted according to the population of West Pomerania.

reported higher serum aldosterone concentration in women [32]. A comparison of subject characteristics shows that both study populations were of European descent. However, there were differences in age and in BMI. Nonhypertensives in Framingham were older (mean age 55 and 56 years for men and women, respectively) and had higher BMI (28.1 and 26.3 kg/m^2 for men and women, respectively) than their SHIP counterparts. Yet, neither age nor BMI can explain the differences in aldosterone. In SHIP, age was not related to PAC, and in both studies women had lower BMI than men. In another study [33], no significant sex differences in PAC were detected. In that study [33], the methodology for PAC measurements was identical to that used in our study, but only 50 subjects (25 women) were examined. For a final explanation of the different study results, further research needs to be done.

Next to our study, six other studies [34–39] provided values for an elevated ARR (▶ **Fig. 3**) based on PAC and PRC, or serum aldosterone concentration and PRC. In these studies the ARR ranged from 32 to 62. These values were obtained predominantly by contrasting the ARR of patients with confirmed PAL to that of hyper- or normotensives without PAL [34,37–39]. In one study the limit for an elevated ARR was chosen arbitrarily [35]. In another study the limit was defined as equivalent of an ARR of 50 determined from PAC to Plasma Renin Activity [36]. Conditions of blood sampling, and assays varied between the studies. None of the other studies is population-based. They were conducted either with patients in primary care [35,36] or in hypertension referral centers [34,37,38]. In the latter, values for an elevated ARR were highest, followed by those from primary care. Our population-based estimates were the lowest. Compared to the other studies, our study cannot give cutoffs for PAL screening, as in SHIP no confirmatory tests were conducted. However, we showed that in the general population an ARR above 14.2 and 22.4, in young and older men, respectively (20.3 and 25.5 in young and older women, respectively) has to be considered as elevated.

Concerning medication, our study confirms the expected effects of various antihypertensives on PAC, PRC, and the ARR. We dem-

Table 3 Median and interquartile ranges for PAC, PRC, and ARR in the non reference population by type of medication compared to the reference population

Type of medication	n		PAC (ng/l)		PRC (ng/l)		ARR	
	Males	Females	Males	Females	Males	Females	Males	Females
Antihypertensives (ATC C02)	3	5	28.0 (74.0)	65.0* (246.0)	21.6 (21.0)	5.8 (16.0)	3.8 (21.1)	15.2* (3.4)
Diuretics (ATC C03)	7	13	60.0 (113.0)	74.0* (79.0)	10.7 (8.2)	10.2* (2.9)	8.6 (3.7)	9.0 (6.7)
Calcium channel blockers (ATC C08)	32	26	72.0 (61.0)	58.0* (29.0)	8.8 (8.9)	6.2 (9.4)	6.0 (4.8)	9.0* (7.8)
Beta blockers (ATC C07)	119	172	43.0* (37.0)	34.0* (31.0)	6.4* (5.8)	4.1* (4.0)	5.7* (6.2)	6.7* (11.2)
Agents acting on the renin-angiotensin system (ATC C09)	113	112	36.0* (31.0)	34.0 (36.0)	23.8* (39.7)	17.2* (24.2)	1.5* (2.1)	1.6* (3.7)
Combination of the above	397	318	43.0 (45.0)	41.0* (38.0)	17.8* (36.8)	9.5* (15.4)	2.3* (5.5)	3.8* (7.4)
Reference population	546	801	47.0 (40.0)	38.0 (36.0)	10.4 (7.9)	7.1 (5.9)	4.9 (4.0)	5.1 (5.9)

PAC: plasma aldosterone concentration; PRC: plasma renin concentration; ARR: aldosterone-to-renin ratio
Continuous data are given as median (interquartile range)
* Statistically significant differences to the reference population
All data were weighted according to the population of West Pomerania

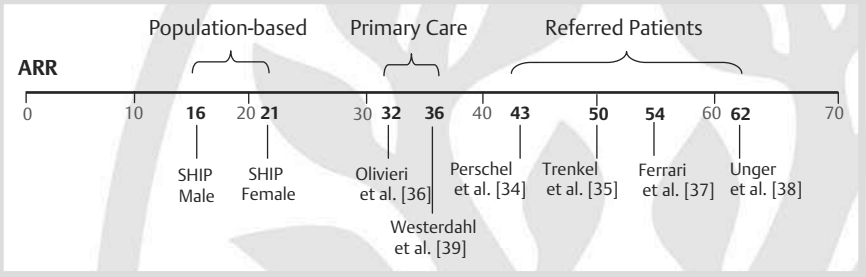

Fig. 3 Comparison of elevated aldosterone-to-renin ratio (ARR) limits in seven studies by type of study population. In all studies plasma renin concentration (PRC) was measured. Units of plasma aldosterone concentration (PAC) and PRC were transformed to ng/l. Unger et al. (2004) [38], Ferrari et al. (2004) [37], and Trenkel et al. (2002) [35] suggest to use elevated ARR in combination with elevated PAC above 200 ng/l in screening for primary hyperaldosteronism (PAL).

onstrate that the intake of beta blockers is associated with lower PRC and higher ARR, while the intake of agents acting on the renin-angiotensin system is associated with higher PRC and lower ARR in both sexes, and with lower PAC in males. As diuretics, antihypertensives, and calcium channel blockers were rarely taken as monotherapy their effects on the ARR were hardly observable in our study and we refrain from interpreting these results.

Also estrogen intake was confirmed to influence the ARR. Women up to 54 years using oral contraceptives or receiving hormone replacement therapy exhibited lower PRC and higher ARR levels. Their upper reference limit for the ARR was therefore higher than that in women not taking estrogens. In women above 54 years estrogen intake was rare, which might explain why results were not statistically significant in this age group.

Variations in PAC and PRC depending on the time of blood sampling were small and not statistically significant. Therefore, we assume that our reference ranges are not biased by the previously described circadian rhythms of aldosterone and renin [26]. Also differences in PAC, PRC, and the ARR between fasting and nonfasting subjects were negligible and not statistically significant. However, subjects were not randomly fasting, which might have biased these results.

The major strength of our study is the population-based sample design, which allows us to provide representative results for north-east Germany. To avoid possible bias due to nonresponse in baseline or drop-out between baseline and follow-up we weighted all data. A comparison of reference values calculated with and without weights showed that this was indeed necessary. We found small differences, in decimal places, in most but not all reference ranges. Major differences were seen in two reference ranges. In females aged 25–54, the reference range for PAC was 6–131 ng/l without, and 5–139 ng/l with weighting. In females aged 55–74, the reference range for PRC was 1.7–19.8 ng/l without, and 1.7–23.5 ng/l with weighting.

We are aware that among our study subjects, noncompliance with intake of self-reported medication might be present, as this is a common problem [40]. Therefore, median values of PAC, PRC, and ARR in the nonreference population might be influenced. Furthermore, for the definition of hypertension repeated measurements of blood pressure on two or more occasions are recommended [41,42]. A re-examination of the blood pressure on a second occasion was, due to high personal and logistic expenses, not possible. However, to avoid a white-coat hypertension effect we performed three subsequent measurements, of which the mean of the second and third were used for statistical analysis. A control for systematic examiner differences was negative. To further ensure high quality blood pressure measurements all digital blood pressure monitors were calibrated before and controlled weekly during data collection.

Our study is restricted to provide reference ranges for PAC, PRC, and the ARR. As confirmatory tests for PAL were not done in SHIP, we cannot estimate the sensitivity and specificity of our upper ARR reference limit for PAL screening. Nevertheless, high PAC and ARR are predictive for the development and severity of hypertension [43,44], regardless whether or not the subjects under investigation suffer from PAL. Transferability of our study results is also limited. Due to the nonstandardized measurement methods and a high variability of PAC and PRC assays, our reference ranges are only valid when similar assays are applied.

In conclusion, we have provided reference ranges for PAC, PRC, and the ARR in a general population. The upper reference limit for the ARR as used in PAL screening is between 14.2 in young men and 25.5 in older women. This is clearly lower than previously reported values from studies in hypertensive patients in primary care or hypertension referral centers. Furthermore, we

confirm that PAC and PRC are associated with various factors, including sex, age as well as intake of estrogens and antihypertensive medication of various classes.

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7.1.2. Association of plasma aldosterone with the metabolic syndrome in two German populations.

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CLINICAL STUDY

Association of plasma aldosterone with the metabolic syndrome in two German populations

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Abstract

Objective: The aim of this study was to analyze the potential association of the plasma aldosterone concentration (PAC) with the metabolic syndrome (MetS) and its components in two German population-based studies.

Methods: We selected 2830 and 2901 participants (31–80 years) from the follow-ups of the Study of Health in Pomerania (SHIP)-1 and the Cooperative Health Research in the Region of Augsburg (KORA) F4 respectively. MetS was defined as the presence of at least three out of the following five criteria: waist circumference $\geq$94 cm (men (m)) and $\geq$80 cm (women (w)); high-density lipoprotein (HDL) cholesterol $<$1.0 mmol/l (m) and $<$1.3 mmol/l (w); blood pressure $\geq$130/85 mmHg or antihypertensive treatment; non-fasting glucose (SHIP-1) $\geq$8 mmol/l, fasting glucose (KORA F4) $\geq$5.55 mmol/l or antidiabetic treatment; non-fasting triglycerides (SHIP-1) $\geq$2.3 mmol/l, fasting triglycerides (KORA F4) $\geq$1.7 mmol/l, or lipid-lowering treatment. We calculated logistic regression models by comparing the highest study- and sex-specific PAC quintiles versus all lower quintiles.

Results: MetS was common with 48.1% (m) and 34.8% (w) in SHIP-1 and 42.7% (m) and 27.5% (w) in KORA F4. Our logistic regression models revealed associations of PAC with MetS, elevated triglycerides, and decreased HDL cholesterol in SHIP-1 and KORA F4.

Conclusions: Our findings add to the increasing evidence supporting a relation between aldosterone and MetS and suggest that aldosterone may be involved in the pathophysiology of MetS and lipid metabolism disorders.

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Introduction

The metabolic syndrome (MetS) is a cluster of multiple metabolic abnormalities, including visceral obesity, impaired glucose homeostasis, dyslipidemia, and hypertension. In the majority of cohort studies, associations of MetS with incident cardiovascular morbidity and mortality were observed (1, 2). Yet, there is an ongoing debate on whether MetS improves cardiovascular risk prediction above its single components (3, 4).

Aldosterone, a steroid hormone, produced in the zona glomerulosa of the adrenal cortex, is suggested to promote the development of MetS (5–7). Excessive levels of circulating aldosterone contribute to hypertension and cardiovascular disease (8). Patients with excessive chronic autonomous aldosterone, known as primary aldosteronism, exhibit a higher prevalence of MetS than patients with essential hypertension (9).

Current findings from cell culture experiments and animal models (10, 11) as well as from epidemiological studies (12) suggest a complex cross talk between the adipose tissue and the adrenal gland. Human adipocytes produce mineralocorticoid-releasing factors that stimulate aldosterone secretion, which in turn promotes adipogenesis (11, 13, 14). Furthermore, epidemiological studies demonstrate that high plasma aldosterone concentrations (PAC) are associated with impaired insulin metabolic signaling, impaired pancreatic β-cell function, and insulin resistance (15, 16). In cross-sectional analyses, associations of aldosterone with MetS were demonstrated (9, 17).

Although the previous cross-sectional studies were restricted to Black individuals (17, 18) or patients with primary aldosteronism (9), we aimed to explore the associations of PAC with MetS in the general population. We applied data from two large, population-based

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German studies: the Study of Health in Pomerania (SHIP), which was conducted in the northeast of Germany and the Cooperative Health Research in the Region Augsburg (KORA), which was conducted in the south of Germany. The two study regions differ with respect to the prevalence of arterial hypertension (19), with higher levels in northeast than in south Germany. Owing to the higher prevalence of hypertension, antihypertensive drugs, which often alter PAC levels, are used more frequently in northeast than in south Germany. In the present analyses, we also considered the influence of PAC-altering drugs (20) on our results.

Materials and methods

Study populations

The investigations in both the studies were carried out in accordance with the Declaration of Helsinki, including written informed consent of all participants. The survey and study methods of both the studies were approved by the institutional review boards (SHIP: ethics committee of the University of Greifswald; KORA: ethics committee of the Bavarian Chamber of Physicians, Munich).

The study of health in Pomerania

SHIP-1 is the first follow-up of the SHIP study, a population-based health survey conducted in the northeast of Germany in the cities of Greifswald, Stralsund, Anklam, and surroundings between 1997 and 2001. A total sample of 7008 subjects was drawn from the target population consisting of all German residents of the region aged 20–79 years. Study design and sampling methods were previously described (21). Of the 4308 participants of the SHIP-0 baseline examination, 3300 participated in the first 5-year follow-up, designated as SHIP-1. Between baseline and follow-up examinations, 234 individuals (5.4%) had deceased and 126 individuals (2.9%) had moved off the study region. Of the remaining 3948 eligible participants, 648 refused to participate in the follow-up, resulting in a response of 83.6%.

Cooperative health research in the region Augsburg

The KORA F4 study is a follow-up of the KORA S4 study, a population-based health survey conducted in the city of Augsburg and two surrounding counties between 1999 and 2001. A total sample of 6640 subjects was drawn from the target population consisting of all German residents of the region aged 25–74 years. Study design and sampling methods were previously described (22). Of all the 4261 participants of the S4 baseline study, 3080 between 31 and 82 years of age also participated in the 7-year follow-up F4 study. Among the persons considered ineligible for F4, 176 (4%) had died in the meantime, 206 (5%) lived outside the study region or were completely lost to follow-up, and 12 (0.2%) had demanded deletion of their address data. Of the remaining 3867 eligible persons, 174 could not be contacted, 218 were unable to come because they were too ill or had no time, and 395 were not willing to participate in this follow-up, giving a response of 79.6%.

Exclusions

To create a comparable age range between SHIP-1 and KORA F4, we excluded all the participants younger than 31 years (SHIP-1, $n=187$) and older than 80 years (SHIP-1, $n=131$; KORA F4, $n=31$). Further exclusion criteria were missing values for PAC (SHIP-1, $n=36$; KORA F4, $n=33$) or for one or more of the components of MetS (SHIP-1, $n=30$; KORA F4, $n=27$), pregnancy or missing data on pregnancy (SHIP-1, $n=16$; KORA F4, $n=12$), type 1 diabetes mellitus (SHIP-1, $n=7$; KORA F4, $n=6$), and renal disease defined as creatinine clearance <50 ml/min (SHIP-1, $n=148$; KORA F4, $n=92$). Some participants were excluded for multiple reasons resulting in overall study populations of 2830 individuals in SHIP-1 and 2901 individuals in KORA F4. In both the studies, drug intake was categorized according to the anatomical therapeutical chemical (ATC) classification index. To evaluate the influence of PAC-altering medication (20) on our results, we repeated all the calculations in participants without intake of antiadrenergic agents (ATC C02), diuretics (ATC C03), β-blockers (ATC C07), calcium channel blockers (ATC C08), or agents acting on the renin–angiotensin system (ATC C09) (SHIP-1, $n=1117$; KORA F4, $n=868$). This resulted in study sub-populations of 1713 individuals in SHIP-1 and 2033 individuals in KORA F4.

Physical examinations

During the physical examination, standardized measurements of height, weight, waist circumference, and blood pressure were performed. In both the studies, systolic and diastolic blood pressures were measured three times on the right arm of the seated participant, using an oscillometric digital blood pressure monitor (HEM-705CP, OMRON Corporation, Tokyo, Japan). For statistical analyses, the mean of the second and third measurements was used. Elevated blood pressure was defined as systolic or diastolic ≥ 130 or 85 mmHg, respectively, or self-reported use of antihypertensive medication. Antihypertensive medication was defined following the guidelines of the German Hypertension Society (SHIP-1: 18th edition (23), KORA F4: 19th edition (24)). Fibrates (ATC C10AB) and nicotinic acids (ATC C10AD) were specified as lipid-lowering drugs. Insulin (ATC A10A) or oral antidiabetics (ATC A10B) were defined as antidiabetic drugs. Intake of estrogens

was defined as oral contraceptives (ATC G03A) or hormone therapy (ATC G03C, G03D, G03F).

Definition of MetS

MetS was defined according to the joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention, National Heart, Lung, and Blood Institute, American Heart Association, World Heart Federation, International Atherosclerosis Society, and International Association for the Study of Obesity (25). MetS was assigned to participants fulfilling at least three out of the following five diagnostic criteria: central obesity, elevated fasting glucose, elevated triglycerides, decreased high-density lipoprotein (HDL) cholesterol, and elevated blood pressure (Table 1). In SHIP-1, blood was collected from mostly non-fasting participants, whereas in KORA F4, blood samples were taken from the participants who had been fasting for at least 10 h. To compensate for the different blood sampling conditions in the two studies, modified cut-off points for elevated glucose and elevated triglycerides were applied. In KORA F4, elevated fasting glucose was defined as serum glucose concentration ≥ 5.55 mmol/l and elevated fasting triglycerides was defined as serum triglyceride concentration ≥ 1.7 mmol/l. As previously established in SHIP-1 (26), elevated non-fasting glucose was defined as serum glucose concentration ≥ 8 mmol/l and elevated non-fasting triglycerides was defined as serum triglyceride concentration ≥ 2.3 mmol/l. These cut-offs were adapted from Lidfeldt *et al.* (27), who used non-fasting blood samples from 6805 middle-aged Swedish women to identify the risk factors for cardiovascular disease and diabetes.

Laboratory measurements

In both the studies, PAC was measured in EDTA plasma, whereas glucose, triglycerides, HDL cholesterol, and potassium were measured in serum.

In SHIP-1, blood samples were taken from the cubital vein of participants in the supine position between 0800 and 2000 h. PAC was measured by an RIA (Coat-A-Count Aldosterone, Siemens Healthcare Diagnostics GmbH, Eschborn, Germany). Inter- and intra-assay coefficients of variation (CV) were 15.7 and 5.4% in low as well as 3.8 and 2.3% in high concentrations respectively (28). Triglycerides and glucose were determined enzymatically (System: Hitachi 717; Reagents: Roche Diagnostics). HDL cholesterol was quantified by lipoprotein electrophoresis (System: HELENA SAS-3 system; Helena 7 BioSciences Europe, Tyne & Wear, UK). Potassium was measured in serum by indirect potentiometry with ion-selective electrodes (QuikLYTE, Dade Behring, Eschborn, Germany).

In KORA F4, blood samples were taken from the cubital vein of seated participants in the morning. PAC was measured with an in-house immunofluorescence assay; inter- and intra-assay CV were 15.2 and 7.3% in low as well as 8.0 and 4.4% in high concentrations respectively (29). Triglycerides and glucose were determined enzymatically (System: Triglycerides: Dimension RxL, Glucose: Hitachi 717; Reagents: Dade Behring, Marburg, Germany). HDL cholesterol was directly quantified by an enzymatic method (System: Dimension RxL; Reagents: Dade Behring). Potassium was measured in serum by indirect potentiometry with ion-selective electrodes (QuikLYTE, Dade Behring).

Statistical analyses

To characterize the study populations, we report means $\pm$ S.D. or medians (first and third quartile) for continuous variables and proportions for categorical variables. Group comparisons were performed using Kruskal–Wallis tests for continuous variables and χ^2 tests for categorical variables. P values <0.05 were considered statistically significant. In order to evaluate the relationship between PAC and MetS, logistic regression models were calculated with MetS and its

Table 1 Definition of MetS in the SHIP-1 and KORA F4 study participants.

Components of MetS[a]	SHIP-1	KORA F4
Elevated waist circumference	Men $\geq$94 cm and women $\geq$80 cm	Men $\geq$94 cm and women $\geq$80 cm
Elevated glucose	Non-fasting glucose: $\geq$8 mmol/l or antidiabetic treatment (ATC A10A, A10B)	Fasting glucose: $\geq$5.55 mmol/l or antidiabetic treatment (ATC A10A, A10B)
Decreased HDL cholesterol	Men $<$1.0 mmol/l, women $<$1.3 mmol/l, or lipid-lowering treatment (ATC C10AB, A10AD)	Men $<$1.0 mmol/l, women $<$1.3 mmol/l, or lipid-lowering treatment (ATC C10AB, A10AD)
Elevated triglycerides	Non-fasting triglycerides: $\geq$2.3 mmol/l or lipid-lowering treatment (ATC C10AB, A10AD)	Fasting triglycerides: $\geq$1.7 mmol/l or lipid-lowering treatment (ATC C10AB, A10AD)
Elevated blood pressure	Systolic or diastolic blood pressures $\geq$130/85 mmHg or self-reported antihypertensive medication[b] in a patient with a history of hypertension	Systolic or diastolic blood pressures $\geq$130/85 mmHg or self-reported antihypertensive medication[b] in a patient with a history of hypertension

ATC, anatomical therapeutical chemical classification index.
[a]MetS was assigned to participants meeting at least three of the five components mentioned in the table.
[b]Antihypertensive medication was defined according to the Guidelines of the German Hypertension Society (SHIP 18th edition (23), KORA 19th edition (24)).

Table 2 Study- and sex-specific 5th quintiles of PAC in SHIP-1 and KORA F4 study populations.

		5th quintile of the PAC distribution (ng/l)	
Study	**Sex**	Including participants taking PAC-altering medication	Excluding participants taking PAC-altering medication
SHIP-1	Males	>75.0	>75.0
	Females	>63.0	>61.0
KORA F4	Males	>58.0	>56.2
	Females	>70.0	>72.0

components as dependent and PAC as independent variables. The SHIP-1 and KORA F4 study populations were divided according to the sex-specific quintiles of the respective PAC distribution (Table 2). Odds ratios and 95% confidence intervals (CI) for the highest versus all lower PAC quintiles are presented.

In a pooled analysis of SHIP-1 and KORA F4 data, we tested the interaction between study region and PAC. Suggestive interactions ($P<0.10$) between study region and PAC were observed in models with MetS, elevated waist circumference, and elevated glucose as dependent variables. Therefore, we performed separate analyses for SHIP-1 and KORA F4.

To address confounding, we adjusted all models for age, sex, potassium, intake of estrogens (in females only), and intake of PAC-raising or PAC-lowering drugs (except for models with elevated blood pressure and MetS as outcome). All logistic regression models were weighted. Weights were accounted for dropouts between baseline and follow-up examinations and are based on socio-demographic and health-related variables. In addition to our main models, we performed a sensitivity analysis applying as alternative cut-off 6.5 mmol/l for elevated fasting glucose in KORA F4. We chose this cut-off according to the American Association of Clinical Endocrinologists, which recommends to define elevated fasting glucose as serum glucose concentration between 110 and 126 mg/dl (6–7 mmol/l) (30). Another sensitivity analysis was performed, applying an alternative cut-off for elevated blood pressure ($\geq$140/90 mmHg), which was used in the 1998 WHO definition for MetS (30). All statistical analyses were performed with SAS 9.1 (SAS Institute, Inc., Cary, NC, USA).

Results

The SHIP-1 and KORA F4 study populations were similar in size with roughly the same proportions of male and female participants. SHIP-1 participants of both sexes had higher body mass index as well as higher systolic and diastolic blood pressure than KORA F4 participants (Table 3). MetS was common in both the study populations with 48.1% in male and 34.8% in female SHIP-1 participants, with 42.7% in male and 27.5% in female KORA F4 participants. Although elevated blood pressure was more frequent in SHIP-1 than in KORA F4, proportions of male and female participants with elevated waist circumference were similar. The proportion of participants who reported a diagnosis of diabetes was higher in SHIP-1 compared with KORA F4. Yet, the proportion of participants with elevated glucose level was higher in KORA F4 (42.7% in men and 27.5% in women) than in SHIP-1 (11.6% in men and 7.9% in women). In our sensitivity analysis with the modified glucose cut-off, proportions of KORA F4 participants with elevated glucose were 13.0% in men and 8.1% in women and thus substantially lower compared with the glucose cut-off applied in the main analysis. We further observed that more than twice as many SHIP-1 than KORA F4 participants had decreased HDL cholesterol, which may be partly due to the use of different methods for HDL cholesterol measurements.

The predominant single components in subjects with MetS were elevated waist circumference and elevated blood pressure. More than 90% of the SHIP-1 and KORA F4 participants with MetS had elevated waist circumference, independent of sex. In SHIP-1, 91% of men and 90% of women with MetS had elevated blood pressure; the respective proportions in KORA F4 were 85% for men and 84% for women.

The proportion of Mets was higher in subjects with PAC levels in the 5th quintile than in subjects with PAC levels in the first to fourth quintiles. Totally, 49.1% of SHIP-1 subjects and 37.7% of KORA F4 subjects with PAC levels in the 5th quintile had MetS, but only 39.4% of SHIP-1 subjects and 34.4% of KORA F4 subjects with PAC levels in the first to fourth quintiles suffered from this metabolic condition.

In our logistic regression models including subjects taking PAC-altering medication, we observed statistically significant associations of high PAC with MetS and elevated triglycerides in both the studies (Table 4). We further detected associations of PAC with elevated waist circumference, elevated glucose, and decreased HDL cholesterol in SHIP-1 but not in KORA F4. In neither of the studies, associations of PAC with elevated blood pressure were observed. The analyses excluding participants taking PAC-altering medication revealed associations of high PAC with MetS, decreased HDL cholesterol, and elevated triglycerides.

Our sensitivity analysis using a modified cut-off for elevated glucose in KORA F4 partly changed our results. In the model including subjects taking PAC-altering medication, we now observed an association of high PAC with elevated glucose (odds ratio 1.65, 95% CI: 1.28–2.12). In the model excluding subjects with PAC-altering medication, no association was observed (odds ratio 1.47, 95% CI: 0.96–2.26). This modified cut-off for elevated glucose provided results more similar to the results from SHIP-1. Our second sensitivity analysis using a modified cut-off for elevated blood pressure also

Table 3 Characteristics of the SHIP-1 and KORA F4 study populations. Values are reported as mean (S.D.) for age, BMI, systolic and diastolic blood pressures, as median (first and third quartile) for PAC, and as proportions for categorical variables.

	Males			Females		
Characteristics	SHIP-1	KORA F_4	*P*	SHIP-1	KORA F_4	*P*
n	1369	1411		1461	1490	
Age (years)	55.2 (13.3)	55.9 (12.9)	0.17	53.6 (12.8)	55.0 (12.6)	<0.01
BMI (kg/m^2)	28.5 (4.2)	28.0 (4.2)	<0.01	27.9 (5.5)	27.4 (5.4)	<0.01
Systolic BP (mmHg)	137.4 (18.5)	127.4 (17.0)	<0.01	128.1 (19.1)	116.8 (17.6)	<0.01
Diastolic BP (mmHg)	84.1 (10.5)	77.7 (10.0)	<0.01	80.3 (9.8)	73.1 (9.2)	<0.01
Diabetes (%)	12.1	7.4	<0.01	9.0	5.6	<0.01
MetS (%)	48.1	42.7	NA	34.8	27.5	NA
Elevated waist circumference (%)	67.3	66.7	0.74	67.6	69.4	0.30
Elevated glucose (%)	11.6	42.4	NA	7.9	24.2	NA
Decreased HDL cholesterol (%)	54.6	15.6	NA	51.5	24.1	NA
Elevated triglycerides (%)	31.9	34.5	NA	16.1	17.2	NA
Elevated BP (%)	74.7	56.8	<0.01	56.5	38.9	<0.01
Intake of						
Antihypertensive drugs (%)	37.0	31.4	<0.01	33.7	26.6	<0.01
PAC-raising drugs (%)[a]	7.5	17.9	<0.01	6.8	15.4	<0.01
PAC-lowering drugs (%)[b]	41.1	31.9	<0.01	37.0	27.7	<0.01
Oral contraceptives (% of women <50 years)	–	–		17.0	17.2	0.92
Systemic hormone therapy (% of women ≥50 years)	–	–		10.8	13.8	0.06
PAC (ng/l)	45.0 (29.0–68.0)	35.2 (24.0–52.0)	NA	38.0 (22.0–58.0)	42.0 (28.0–62.6)	NA

BP, blood pressure; PAC, plasma aldosterone concentration, NA, not applicable. Definitions of MetS and its components as referred to in Table 1. Definition of diabetes: self-reported physicians diagnosis. Differences between SHIP-1 and KORA F4 were assessed by Kruskal–Wallis test (continuous variables) and χ^2 tests (categorical variables). *P* values <0.05 were considered significant. Owing to different sampling conditions as well as different laboratory methods, group comparisons were not performed for MetS, elevated glucose, decreased HDL cholesterol, elevated triglycerides, and PAC.
[a]PAC-raising drugs: diuretics (ATC C03).
[b]PAC-lowering drugs: antiadrenergic agents (ATC C02), β-blockers (ATC C07), calcium channel blockers (ATC C08), and agents acting on the renin–angiotensin system (ATC C09).

changed our results. With the new definition, 57.6% of men and 43.9% of women in SHIP-1 as well as 42.6% of men and 31.5% of women in KORA F4 had elevated blood pressure. In SHIP-1, we now observed significant associations between high PAC and elevated blood pressure (model including subjects with PAC-altering medication: odds ratio 1.24, 95% CI: 1.04–1.48; model excluding subjects with PAC-altering medication: odds ratio 1.31, 95% CI: 1.03–1.66). In KORA F4, no association was observed.

Discussion

We observed associations of PAC with MetS and lipid metabolism disorders in two studies of non-selected volunteers conducted in northeast and south Germany. Previous reports from cross-sectional studies demonstrated associations of aldosterone with MetS in Black population (17, 18) and a higher prevalence of MetS in primary aldosteronism than in essential hypertension (9). In a longitudinal analysis (7), using data from the

Table 4 Associations between high PAC with MetS and its components. Definitions of MetS and its components as referred to in Table 1. Odds ratios and 95% confidence intervals (CI) for fifth versus first to fourth PAC quintiles are displayed. Models were calculated separately for SHIP-1 and KORA F4. All logistic regression models were adjusted for age (continuous), sex (male/female), potassium (continuous), and intake of estrogens (in females only). Models including all subjects were further adjusted for intake of PAC-raising or PAC-lowering drugs (except for models with elevated blood pressure or MetS as outcome).

			Components of the MetS				
Model	***n***	**MetS**	Elevated waist circumference	Elevated glucose	Decreased HDL cholesterol	Elevated triglycerides	Elevated blood pressure
SHIP-1							
OR (95% CI)	2830	1.64 (1.38–1.95)	1.71 (1.41–2.09)	1.49 (1.14–1.95)	1.34 (1.13–1.58)	1.47 (1.22–1.77)	1.11 (0.93–1.34)
KORA F4							
OR (95% CI)	2901	1.38 (1.15–1.64)	1.07 (0.89–1.29)	1.10 (0.92–1.33)	1.08 (0.89–1.31)	1.27 (1.06–1.52)	1.15 (0.96–1.38)
SHIP-1 excluding participants with PAC-altering medication							
OR (95% CI)	1713	1.51 (1.19–1.91)	1.54 (1.23–1.93)	1.24 (0.67–2.30)	1.36 (1.10–1.69)	1.42 (1.09–1.84)	1.09 (0.87–1.37)
KORA F4 excluding participants with PAC-altering medication							
OR (95% CI)	2033	1.28 (1.01–1.62)	0.99 (0.81–1.21)	0.95 (0.75–1.22)	1.29 (1.01–1.65)	1.35 (1.08–1.70)	1.00 (0.80–1.26)

Framingham Heart Study, aldosterone was associated with incident MetS. Our results add to the increasing evidence supporting a relation between aldosterone and MetS.

The biological mechanisms underlying the association of aldosterone with MetS are not yet fully understood. It has been suggested that elevated circulating aldosterone levels may cause increased inflammation and oxidative stress, which in turn may promote insulin resistance, impaired pancreatic β-cell function, endothelial dysfunction, and hypertension (31). Findings from observational studies support the evidence from experimental studies. In White, predominantly hypertensive individuals' relationship between PAC on the one hand and insulin, insulin resistance, and hyperinsulinemia on the other hand were observed (32). In Black subjects, an association of PAC with insulin and the homeostatic model assessment insulin resistance index was detected (18). Yet, in the same study (18), no association between PAC and glucose was observed. Our regression models revealed an association between PAC and elevated non-fasting glucose. We also observed an association between PAC and elevated fasting glucose when we applied the 6.5 mmol/l cut-off, but not when we applied the weaker 5.55 mmol/l cut-off. Both associations were lost in models excluding subjects taking PAC-altering medication. As in these models only 2.7% of SHIP-1 and 4.5% of KORA F4 participants had elevated glucose, this might be a power problem. Whether there are associations of PAC with insulin in SHIP-1 or KORA F4 was not addressed, since we currently do not have any data on insulin concentrations from either study.

Consistent with our findings, associations of PAC with the obesity-related components of MetS including decreased HDL cholesterol (17), elevated triglycerides (18), and elevated waist circumference (17, 18) were observed in Black subjects. It has been suggested that in a self-strengthening process, human adipocytes may stimulate aldosterone production by producing mineralocorticoid-releasing factors, which in turn can promote adipogenesis and inflammation (13, 14). Our analyses revealed associations of high PAC with MetS, elevated triglycerides, and decreased HDL cholesterol in SHIP-1 and KORA F4 as well as elevated waist circumference only in SHIP-1. The corresponding odds ratios of all associations were higher in SHIP-1 than in KORA F4. Previous studies (19, 33) demonstrated that the prevalence of cardiovascular risk factors such as smoking or obesity vary between the SHIP-1 and the KORA F4 populations. We assume that the higher burden of cardiovascular and metabolic risk factors in northeast compared with south Germany partially accounts for the higher strength of the associations of PAC with the components of MetS in SHIP-1 versus KORA F4.

We further assume that our results have been affected by the intake of PAC-altering medication, as in all models, except HDL cholesterol, the strength of the associations decreased when subjects taking PAC-altering medication were excluded versus included. At the same time, the associations observed in the models excluding versus including subjects taking PAC-altering medication were essentially the same. This implies that independent of whether PAC levels are altered by drug intake, high PAC levels are associated with MetS and its components.

A further conceivable explanation for the differences between SHIP-1 and KORA F4 may be unaddressed confoundingly. Salt intake, for instance, affects both PAC and blood pressure (34). Unfortunately, salt intake was not measured in our cohorts. Furthermore, genetic variations between the two populations (35) may have influenced our results.

The contribution of excessive aldosterone levels to hypertension is unquestioned (8, 36). Results from two longitudinal analyses using data from North America and France (37, 38) demonstrated that serum aldosterone levels may contribute to the development of hypertension. Other cross-sectional studies suggest that the association of aldosterone with elevated blood pressure is generally stronger in Black than in White individuals (39) or is only present in older individuals (mean age 61 years) (17). In contrast to these studies (17, 39), our cross-sectional analyses did not reveal a significant association between PAC and elevated blood pressure, which may have different reasons. First, in the model excluding subjects taking PAC-altering medication, the excluded participants represent the majority of participants with elevated blood pressure (55.5%) and MetS (58.1%). This introduces a selection in the study population and reduces statistical power. Secondly, we observed no association between high PAC and elevated blood pressure defined as systolic or diastolic $\geq$130/85 mmHg. When elevated blood pressure was defined as systolic or diastolic $\geq$140/90 mmHg, we observed an association in SHIP-1. This might imply that the association of high PAC and elevated blood pressure increases with severity of disease.

The reported prevalence of MetS in the SHIP-1 and KORA F4 study populations were impressively high. In a previous publication (40) using KORA data, it was shown that the prevalence of MetS strongly depends on the criteria used to define the condition. In a population of elderly KORA participants, the prevalence of MetS varied between 24–46% in women and 28–57% in men, depending on the definition used (40). We, therefore, applied a recent definition for MetS, published by several major organizations, which represents an attempt to harmonize MetS (25).

Major strengths of our study are the population-based study design, the large number of participants in both studies and the standardized data collection performed by trained and certified examiners.

Limitations arise from technical differences in the measurements of PAC, plasma glucose, triglycerides,

and HDL cholesterol between SHIP-1 and KORA F4. Prior to the present analyses, we examined the influence of blood sampling time and fasting status on PAC and found no significant effect (39). By creating study-specific quintiles for PAC and using modified cut-offs for non-fasting levels in the definition of MetS, we corrected for these differences. Nevertheless, we cannot rule out that the different methods used to measure PAC, plasma glucose, HDL cholesterol, or triglycerides in the two studies introduced some bias. Another limitation of our study is that all participants were under random sodium diet and under regular medication, including PAC-altering drugs. In contrast to clinical studies with small samples, SHIP-1 and KORA F4 are large, observational studies, in which it is not possible to change antihypertensive treatment or dietary habits of our participants. Moreover, we used single-occasion measurements from our participants. It is arguable whether single measurements can appropriately represent the PAC, plasma glucose, HDL cholesterol, or triglyceride profiles of our subjects. Unfortunately, for logistic reasons, it is impossible to repeat blood sampling in our population-based studies.

Taken together, our results obtained in non-selected, White individuals from northeast or south Germany revealed associations of PAC with MetS and lipid metabolism disorders. Owing to the high prevalence of MetS and its components in the general population, future research should focus on a better understanding of the mechanism underlying the associations of PAC with MetS and lipid metabolism disorders.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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7.1.3. Plasma aldosterone levels and aldosterone-to-renin ratios are associated with endothelial dysfunction in young to middle-aged subjects.

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Plasma aldosterone levels and aldosterone-to-renin ratios are associated with endothelial dysfunction in young to middle-aged subjects

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ABSTRACT

Objective: Small clinical studies suggested a role for aldosterone in the development of endothelial dysfunction. We investigated whether the plasma aldosterone concentration (PAC) or the aldosterone-to-renin ratio (ARR) were associated with decreased endothelial function as measured by flow-mediated dilation (FMD) of the brachial artery in the general population.
Methods: Our study population comprised 972 participants from the Study of Health in Pomerania, who were not treated with antihypertensive medication. We performed age-stratified (<50 and ≥50 years) ordinal logistic regression analyses. FMD was categorised as decreased (1st quintile), moderate (2nd–4th quintile), or increased (5th quintile). PAC and ARR were divided into low, moderate, and high values according to age- and sex-specific tertiles. All models were re-calculated for 871 subjects with PAC and ARR within the study-specific reference ranges. Odds ratios (OR) and 95% confidence intervals (CI) are presented.
Results: Subjects <50 years with high PAC (OR 1.60; 95% CI 1.07–2.38) or ARR (OR 1.81; 95% CI 1.21–2.73) had higher odds for decreased FMD than subjects with low PAC or ARR, respectively. Similar results were obtained in analyses restricted to subjects with PAC and ARR within the reference range. High-normal PAC (OR 1.62; 95% CI 1.07–2.47) or ARR (OR 1.62; 95% CI 1.05–2.50) was associated with higher odds for decreased FMD when compared with low-normal PAC or ARR, respectively. These associations were not observed in subjects ≥50 years.
Conclusions: High and high-normal PAC or ARR contribute to an impaired FMD and subsequently the progression of subclinical atherosclerosis in young to middle-aged subjects.

1. Introduction

Impaired endothelial function represents an early stage of arterial wall damage and is a surrogate marker of subclinical atherosclerosis [1–3]. Characteristics of endothelial dysfunction include alterations in vascular tone, impaired endogenous endothelial repair, increased vascular inflammation, and thrombosis [4].

Established cardiovascular risk factors, such as obesity or diabetes mellitus, are major causes of endothelial dysfunction. Furthermore, age-dependent impairment of endothelial function has been reported [5–7] and is assumed to be secondary to the diminished bioavailability of nitric oxide and the accumulation of cardiovascular risk factors throughout life [8–10]. Impaired function of the vascular endothelium may reflect individual predisposition to arterial hypertension, which may subsequently cause endothelial dysfunction [11].

Small clinical studies [12,13] of patients with primary aldosteronism suggested a key role for aldosterone excess in the development of endothelial dysfunction as assessed by flow-mediated dilation (FMD) of the brachial artery. Hypertensive patients with primary aldosteronism had significantly lower FMD values than those without primary aldosteronism [13]. Furthermore, surgical or pharmacological treatment of primary aldosteronism significantly enhanced FMD [12,13]. Another study in 130 volunteers found an impairment of nitric oxide-mediated

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dilation in hypertensive subjects with low-renin status or high aldosterone-to-renin ratios (ARR) as compared to normotensives [14].

While it is generally accepted that endothelial function is impaired in primary aldosteronism, there is little known about the associations between the plasma aldosterone concentration (PAC) or the ARR and endothelial function in the general population. Thus, the aim of our study was to determine whether PAC or ARR were associated with decreased brachial artery FMD in a large population-based cohort. Furthermore, we investigated the associations of PAC or ARR in a subset of our study population that had PAC and ARR within the study-specific reference ranges.

2. Materials and methods

2.1. Study population

The Study of Health in Pomerania (SHIP) is a longitudinal population-based study in northeastern Germany. The study design and sampling methods have been previously described [15]. Briefly, 4308 men and women from a representative population sample of 7008 subjects participated in baseline examinations between October 1997 and May 2001. Five years later, between March 2003 and July 2006, the first follow-up examinations (designated as SHIP-1) were conducted in 3300 participants. All participants gave written, informed consent. The study conformed to the principles of the Declaration of Helsinki as reflected by an *a priori* approval of the Ethics Committee of the Board of Physicians Mecklenburg-West Pomerania at the University of Greifswald.

Sociodemographic and behavioural characteristics of study participants were assessed by personal interviews. The participants' medication was categorised according to the anatomical–therapeutic–chemical (ATC) classification code. During the physical examination, standardised measurements of height, weight, waist circumference, and blood pressure were performed. Systolic and diastolic blood pressures were measured three times on the right arm of the seated participant using a digital blood pressure monitor (HEM-705CP, OMRON Corporation, Tokyo, Japan). For statistical analyses, the mean of the second and third measurements was used.

FMD measurement was offered to all SHIP-1 participants, and 1692 subjects opted to receive this examination. A median time of 16.5 days (1st–3rd quartile ranged from 0 to 75 days) passed between basic examination and FMD measurement. Following a standardised protocol, certified examiners performed the FMD measurement in response to post-ischemic forearm hyperaemia using a 10 MHz linear array transducer (Cypress Acuson, Siemens, Erlangen, Germany). Details on the study protocol and quality assurance procedures have been described elsewhere [16]. Here, we present FMD as percent dilation occurring during the maximum response normalised to baseline brachial artery diameter. Of the 1692 FMD participants, we excluded 174 because of low-quality of sonographic images. We further excluded subjects with renal insufficiency (defined as creatinine clearance <50 ml/min, $n = 21$), pregnant women ($n = 4$), and participants with missing PAC or ARR values ($n = 11$). We further excluded 508 participants taking PAC-, ARR- or FMD-altering medication, including antiadrenergic agents (ATC C02), diuretics (ATC C03), beta blockers (ATC C07), calcium channel blockers (ATC C08), agents acting on the renin-angiotensin system (ATC C09), peripheral vasodilators (mainly purine derivatives, ATC C04), and vasodilators used in cardiac diseases (mainly nitrates, ATC C01D). An additional two subjects were excluded due to insufficient information on confounders. Our final study population comprised 972 subjects.

For our analyses in the subgroup of subjects with normal PAC and ARR, we further excluded all subjects with PAC, plasma renin concentration (PRC) or ARR outside the previously established study-specific reference ranges ($n = 101$) [17]. This resulted in a study population of 871 subjects.

2.2. Laboratory measurements

For SHIP-1, blood samples were taken between 8:00 a.m. and 7:30 p.m. from the cubital vein of non-fasting subjects in the supine position. Potassium was measured in serum by indirect potentiometry with ion-selective electrodes (QuikLYTE, Dade Behring, Eschborn, Germany). Hypokalemia was defined as serum potassium concentrations <3.5 mmol/l. PAC and PRC were measured in EDTA plasma (PAC was measured with Coat-A-Count Aldosterone, Siemens Healthcare Diagnostics, Eschborn, Germany; and PRC was measured with Renin III Generation, Cisbio Bioassay, Bagnols-sur-Cèze Cedex, France), as previously reported [17].

2.3. Statistical analyses

To describe the study population, we report medians (1st, 3rd quartile) for continuous variables and proportions for categorical variables. For group comparisons, we used Kruskal–Wallis and Chi-squared tests. p values <0.05 were considered statistically significant.

Given the lack of standardised and generally accepted cut-offs for normal FMD [18], we applied a distribution-based definition to categorise FMD. FMD in the lowest age- (25–34, 35–44, 45–54, 55–64, and over 64 years of age) and sex-specific quintile ($n = 196$) was defined as decreased, FMD in the 2nd–4th quintiles was defined as moderate ($n = 583$), and FMD in the highest quintile ($n = 193$) was defined as increased.

Ordinal logistic regression using the proportional odds model was used to examine the associations of PAC or ARR with FMD in the whole study population, as well as in subjects with PAC and ARR within the reference ranges. In these models, a single odds ratio (OR) was calculated that represents the association of a predictor variable with all higher vs. lower risk combinations of the outcome variable categories. In our case the ordinal outcome has three levels (decreased, moderate, and high FMD). The two resulting higher vs. lower risk combinations are: (1) decreased and moderate vs. high FMD and (2) decreased vs. moderate and high FMD. To confirm the validity of the ordered logistic regression model, we tested the proportional odds assumption using a Chi-squared score test, which indicated that the assumption was valid.

The predictor variables (PAC and ARR) entered the model as continuous or categorical variables. PAC and ARR were categorised as low, moderate or high according to the age- (25–34, 35–44, 45–54, 55–64, and over 64 years of age) and sex-specific tertiles of the respective distribution.

In addition to the ordinal regression model we calculated a quantile regression model [19] to explore the changes in median FMD as a function of PAC or ARR. In these models, FMD as well as PAC and ARR were used as continuous variables. As the quantile regression analysis does not require normal distribution of the data, a transformation of the dependent variable was not necessary. Coefficient estimates for the median with 95% confidence intervals (CI) are presented.

Previous studies [5–7,9,10] demonstrated that FMD decreases with age. In older subjects with already impaired endothelial function, high PAC or ARR may not have the same effects as in younger subjects with normal endothelial function. We tested whether the participants' age (younger than 50 years of age vs. 50 years or older) was a potential effect modifier in our ordinal regression models. In three out of four models significant interactions ($p < 0.10$) with the

Table 1
Characteristics of the study population.

Characteristics	Age <50 years	Age ≥50 years
N	566	406
Women (%)	51.6	49.5
Age (years)	39.0 (34.0–44.0)	59.0 (54.0–64.0)*
Systolic BP (mmHg)	122.0 (112.5–133.5)	132.5 (122.0–144.0)*
Diastolic BP (mmHg)	80.0 (74.5–86.0)	83.0 (76.0–89.5)*
Waist circumference (cm)	85.8 (77.0–95.5)	92.4 (84.1–100.0)*
Hypertension (%)	20.9	38.2*
Current smokers (%)	38.7	20.9*
Physical activity (%)	47.7	44.3
Diabetes mellitus (%)	0.7	4.9*
Hypokalemia (%)	0.2	0.0
Potassium (mmol/l)	4.34 (4.12–4.57)	4.35 (4.14–4.60)
FMD (%)	5.6 (3.2–8.6)	3.9 (2.1–6.3)*
PAC (ng/l)	43.0 (26.0–62.0)	41.0 (24.0–58.0)
PRC (ng/l)	8.4 (5.5–12.8)	7.3 (4.9–10.1)*
ARR	5.0 (2.9–8.0)	5.3 (3.1–9.5)*

BP, blood pressure; FMD, flow-mediated dilation of the brachial artery; PAC, plasma aldosterone concentration; PRC, plasma renin concentration; ARR, aldosterone-to-renin ratio.
To convert PAC in pmol/l multiply by 2.775. To convert PRC in mU/l multiply by 1.66. Continuous variables are given as median (1st–3rd quartile), and dichotomous variables are given as percentages.
* $p < 0.05$.

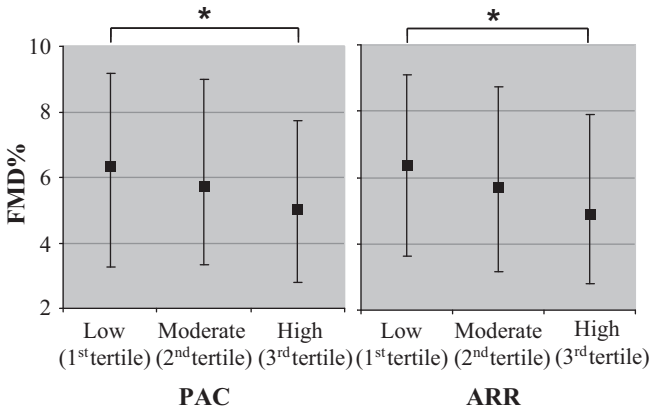

Fig. 1. Median (1st, 3rd quartile) levels of FMD according to tertiles of PAC and ARR in subjects younger than 50 years of age. * $p < 0.05$; FMD, flow-mediated dilation of the brachial artery; PAC, plasma aldosterone concentration; ARR, aldosterone-to-renin ratio.

exposure variable were detected. We therefore decided to perform age-stratified analyses in subjects younger than 50 years of age and in subjects 50 years or older.

All models were adjusted for sex (male, female), age (in years), systolic and diastolic blood pressure (in mmHg), waist circumference (in cm), smoking (yes, no), diabetes mellitus (yes, no), physical activity (yes, no), and time (in days) between blood sampling and FMD measurement. In a sensitivity analysis we further adjusted all models for time of blood sampling. OR and 95% CI for all models are presented. All statistical analyses were performed with SAS 9.1 (SAS Institute Inc., Cary, NC, USA).

3. Results

Systolic and diastolic blood pressures, waist circumference, as well as proportions of hypertension and diabetes mellitus were significantly higher in subjects 50 years of age or older than in subjects younger than 50 years of age. We observed lower ARR as well as higher FMD and PRC in the younger than in older subjects (Table 1).

We detected higher systolic and diastolic blood pressures and higher proportions of hypertension in subjects with high ARR than in subjects with low ARR. Differences in blood pressure levels and proportions of hypertension were not detected between the PAC tertiles. Furthermore, proportions of current smokers and physically active subjects did not significantly vary between the PAC or ARR tertiles (see Supplementary Material for details).

Descriptive statistics further revealed that subjects younger than 50 years of age with high PAC or ARR had significantly lower FMD than subjects with low PAC or ARR (Fig. 1). These observations were not confirmed in subjects 50 years of age or older.

In fully adjusted ordinal regression models, subjects younger than 50 years of age with high PAC had higher odds for decreased FMD than subjects with low PAC (Table 2). Likewise, subjects younger than 50 years of age with moderate or high ARR had higher odds for decreased FMD than subjects with low ARR. When ARR entered the model as a continuous variable, we observed higher odds for decreased FMD with every increase of one standard deviation in ARR. Following the proportional odds model, these associations hold over the three FMD categories (from decreased to increased FMD). Similar results were obtained when the analyses were restricted to subjects with PAC and ARR within the reference ranges. Subjects younger than 50 years of age with high-normal PAC had higher odds for decreased FMD than subjects with low-normal PAC. Subjects with high-normal ARR had higher odds for decreased FMD than subjects with low-normal ARR. We further observed higher odds for decreased FMD with every increase of one standard deviation of ARR. When PAC entered the model as a continuous variable, statistical significance was barely missed. There was no relation between PAC or ARR and FMD in subjects 50 years of age or older. For our sensitivity analysis we additionally adjusted all models for time of blood sampling, which yielded the same results as in our main analyses (data not shown).

Our fully adjusted quantile regression model revealed a significant association between ARR and FMD in subjects younger than 50 years of age. A one unit increase in ARR resulted in a 0.09 decrease in median FMD [coefficient estimate −0.09 (95% CI −0.16, −0.02)]. We found no statistically significant association between PAC and FMD. These findings confirm the results obtained with the ordinal regression analyses with PAC and ARR as continuous variables.

4. Discussion

High and high-normal PAC or ARR were associated with decreased FMD in subjects from the general population. Moreover, we observed a linear association between ARR and FMD but not between PAC and FMD. All detected associations were present in young to middle-aged subjects below the age of 50 years but not in older subjects. The lack of associations in older subjects may be explained by the accumulation of cardiovascular risk factors throughout life. The accumulating risk factors may enforce functional and structural changes in the vascular endothelium, trigging endothelial dysfunction and preceding the development of atherosclerotic disease [1,5,20]. Furthermore, increased age alone, independent of atherosclerotic disease, may lead to vascular dysfunction [5,6]. In older subjects whose vessels are stiffer and endothelial function is already reduced, high PAC or ARR may not have the same effects on FMD as in younger subjects. This might explain our observations of associations of PAC and ARR with decreased FMD as a subclinical phenotype in young to middle-aged subjects only.

The adverse impact of high PAC or ARR on the endothelium was previously demonstrated in patients with primary aldosteronism [12,13]. FMD was significantly lower in hypertensive patients with primary aldosteronism compared to those without primary aldosteronism [12,13]. Furthermore, FMD increased after surgical

Table 2
Odds ratios and 95% confidence intervals for decreased FMD by age-group in the whole study population and in a subgroup of subjects with PAC and ARR within the reference range.

Model[a]	Categorization of PAC and ARR	Age <50 years		Age ≥50 years	
		PAC	ARR	PAC	ARR
All subjects (n = 972)	Moderate vs. low	1.12 (0.74–1.66)	1.64 (1.10–2.46)	0.83 (0.51–1.37)	0.80 (0.50–1.30)
	High vs. low	1.60 (1.07–2.38)	1.81 (1.21–2.73)	1.01 (0.63–1.61)	0.88 (0.54–1.41)
	Continuous (unit = 1SD)	1.14 (0.96–1.34)	1.22 (1.04–1.44)	0.97 (0.79–1.19)	0.94 (0.77–1.16)
PAC and ARR within the reference range (n = 871)	Moderate vs. low	1.07 (0.70–1.63)	1.51 (0.99–2.31)	1.00 (0.59–1.69)	1.04 (0.62–1.74)
	High vs. low	1.62 (1.07–2.47)	1.62 (1.05–2.50)	1.15 (0.70–1.88)	1.11 (0.67–1.84)
	Continuous (unit = 1SD)	1.17 (0.97–1.40)	1.22 (1.02–1.45)	1.09 (0.88–1.35)	1.11 (0.90–1.37)

FMD, flow-mediated dilation of the brachial artery; PAC, plasma aldosterone concentration; ARR, aldosterone-to-renin ratio.
FMD was categorized in decreased (1st quintile), moderate (2nd–4th quintile), or increased (5th quintile). PAC and ARR entered the models as continuous or categorical variables (low 1st tertile, moderate 2nd tertile, and high 3rd tertile).
All models were adjusted for sex (male, female), age (in years), systolic and diastolic blood pressure (in mmHg), waist circumference (in cm), smoking (yes, no), diabetes mellitus (yes, no), physical activity (yes, no), and time between blood sampling and FMD measurement (in days).
[a] Ordinal logistic regression using the proportional odds model.

or pharmacological treatment of primary aldosteronism [12,13]. Also in hypertensive patients without primary aldosteronism, associations between renin activity or ARR and FMD were reported [14]. Duffy and colleagues [14] found that a low renin status or high ARR, but not PAC, were associated with impaired responses to methacholine in hypertensive subjects [14]. Associations of plasma renin with brachial artery FMD were also found in hypertensive and normotensive subjects in the Framingham Heart Study [21]. No association was found between serum aldosterone and FMD [21]. In our study, high PAC was associated with decreased FMD in young individuals but there was no linear association between PAC and FMD. Then again, we identified a linear association between ARR and FMD. This suggests that relative PAC values, seen in relation to PRC, may be more closely linked to FMD than absolute PAC values. Unfortunately, in the report analysing data from the Framingham Heart Study [21], the association between ARR and FMD was not investigated.

Strengths of our study include the various quality assurance methods employed and the large study population. These methods included periodic certification processes of readers and examiners before and during data collection [15,16], ensuring high-quality FMD measurements. However, four limitations of our investigation merit comment. First, due to voluntary participation, the subsample of FMD participants is not representative of the whole SHIP population. FMD participants were younger and probably healthier than non-participants. Second, although the median time interval between blood sampling and FMD measurement was only 16.5 days, it was longer than 90 days in about 20% of our participants. This long interval in a fraction of our subjects may have introduced bias. Third, circadian rhythms with peak secretion in the mornings were described for the aldosterone and renin secretion [22,23]. As in SHIP blood samples were taken at varying time points during day, we recalculated all ordinal regression models adjusted for the time of blood sampling. Since our results remained unchanged, we do not suspect a relevant influence of the circadian secretion on our results. Fourth, our epidemiological study cannot provide causal explanations for the observed associations between PAC or ARR with FMD.

5. Conclusion

In summary, we identified associations of high and high-normal PAC or ARR with decreased FMD in young to middle-aged subjects from the general population. Our data show that high and high-normal PAC or ARR contribute to an impaired FMD and subsequently the progression of subclinical atherosclerosis in young to middle-aged subjects.

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Conflict of interest

There are no conflicts of interest that could be perceived as prejudicing the impartiality of the research reported.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.atherosclerosis.2011.09.008.

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7.2. Publikationsverzeichnis

7.2.1. Originalarbeiten

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7.2.2. Buchbeiträge

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7.3. Eidesstattliche Erklärung

Ich erkläre, dass ich die vorliegende Dissertation selbständig verfasst und keine anderen als die angegebenen Hilfsmittel benutzt habe.

Die Dissertation ist bisher keiner anderen Fakultät, keiner anderen wissenschaftlichen Einrichtung vorgelegt worden.

Ich erkläre, dass ich bisher kein Promotionsverfahren erfolglos beendet habe und dass eine Aberkennung eines bereits erworbenen Doktorgrades nicht vorliegt.

Greifswald, den _____

Anke Hannemann

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