

Aus dem Institut für Klinische Chemie und Laboratoriumsmedizin

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der Universitätsmedizin der Ernst-Moritz-Arndt-Universität Greifswald

**Sexualhormone bei Frauen und deren Assoziation zu kardiovaskulärer
Morbidity und Mortalität**

Inaugural-Dissertation

zur

Erlangung des akademischen Grades

Doktor der Naturwissenschaften in der Medizin

(Dr. rer. med.)

der

Universitätsmedizin

der

Ernst-Moritz-Arndt-Universität

Greifswald

2016

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Tag der Disputation: 06.04.2017

Ausgabe: Elektronische Version - April 2017

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ABKÜRZUNGSVERZEICHNIS

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ATC	Anatomisch-therapeutisch-chemische Klassifikation
AD	Androstendion
BMI	Body Mass Index
DHEA	Dehydroepiandrosteron
DHEAS	Dehydroepiandrosteron-Sulfat
E1	Estron
E2	Estradiol
FAI	Freier Androgenindex
FT	Freies Testosteron
HDL	High-density Lipoprotein
HT	Hormontherapie
IMD	Intima Media Dicke
IVS	Interventrikuläre Septumdicke
KVK	Kardiovaskuläre Krankheiten
LDL	Low-density Lipoprotein
LVDD	Linksventrikulärer end-diastolischer Diameter
LVDS	Linksventrikulärer end-systolischer Diameter
LVH	Linksventrikuläre Hypertrophie
LVPWD	Posteriore Wanddicke
MetS	Metabolisches Syndrom
RWD	Relative Wanddicke
SHBG	Sexualhormon-bindendes Globulin
SHIP	Study of Health in Pomerania
T	Gesamt-Testosteron
T2DM	Typ 2 Diabetes mellitus

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ABBILDUNGSVERZEICHNIS

- Abbildung 1** Biosynthese ausgewählter Sexualhormone.
- Abbildung 2** Überblick der Zusammenhänge zwischen Sexualhormonen/Sexualhormon-bindendem Globulin und kardiovaskulären Risikofaktoren/Mortalität bei Frauen.
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TABELLENVERZEICHNIS

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1. Einleitung

1.1 Metabolismus und Funktion der Sexualhormone

Sexualhormone sind Steroidhormone, die eine zentrale Rolle bei der Geschlechtsentwicklung, der Aktivität der Keimdrüsen, dem Wachstum und der geschlechtsspezifischen Prägung im sexuell-dimorphen Nukleus der präoptischen Region des Gehirns spielen (1). Des Weiteren wirken sie sowohl auf den Knochenstoffwechsel und das Immunsystem, sowie auf die Gefäßhämodynamik (2). Steroidhormone werden in verschiedenen Zellen und Zellkompartimenten durch eine Reihe zeitlich regulierter und gewebsspezifisch exprimierter, steroidogener Enzyme metabolisiert, wobei das polyzyklische Steran das Grundgerüst der Steroidhormone bildet. Aus diesem wird über die Zwischenstufe des Cholesterols die gemeinsame Vorstufe aller Steroidhormone gebildet, das Pregnenolon. Dieses wird sowohl in Androgene (Dehydroepiandrosteron (DHEA), Androstendion (AD), Testosteron) als auch in Estrogene (Estron (E1), Estradiol (E2)) verstoffwechselt (Abbildung 1). Synthetisiert werden die Sexualhormone bei der Frau hauptsächlich in den Gonaden, genauer in den Thekla-Zellen, die um die weiblichen ovariellen Follikel liegen. Zusätzlich bildet die Zona reticularis der Nebenniere Androgene, die nicht gespeichert, sondern direkt freigesetzt werden. Reguliert wird die Produktion über eine Feedback-Inhibition der hypothalamisch-hypophysär-gonadotropen Achse (1). Die Konzentration der Steroidhormone im Blut wird durch zwei geschwindigkeitsbestimmende Schritte ihrer Synthese geregelt. Das ist zum einen die Hydrolyse der Vorratsform des Cholesterols im Zytosol durch eine Esterase und zum anderen der Import des Cholesterols in die Mitochondrien (3). Sowohl die nukleären Androgen- als auch die Estrogenrezeptoren wirken als Transkriptionsfaktoren und wurden in einer Vielzahl von Geweben nachgewiesen, wodurch sich die Bandbreite der endokrinen und kardiometabolischen Effekte von Sexualhormonen auf verschiedenste Körpersysteme der Frau erklärt (2). Nur ein kleiner Teil der im Blut zirkulierenden Sexualhormone liegt ungebunden vor und kann an diese Rezeptoren binden. Der andere, wesentlich größere Teil, ist an die Transportproteine Albumin und an das in der Leber gebildete Sexualhormon-bindende Globulin (SHBG) gebunden (1). An SHBG gebundene Sexualhormone sind allerdings nicht völlig inaktiv, sondern in der Lage, endozytische Wege zu passieren (4) oder freie Sexualhormonkonzentrationen zu regulieren (5). Bezuglich des Katabolismus werden die Sexualhormone durch Biotransformationen in der Leber inaktiviert und über die Niere oder die Galle ausgeschieden.

Bei Frauen erhält besonders die Fluktuation der Hormonkonzentrationen über die Lebensdauer eine Schlüsselrolle in der Betrachtung der Sexualhormone. Während alle

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Sexualhormone und SHBG (im Folgenden: Sexualhormone/SHBG) eine Altersabhängigkeit aufweisen (6, 7), verändern sich die Estrogenkonzentrationen zusätzlich während des Menstruationszyklus, der Gestation und während der Menopause. Nach der Menopause befinden sich die E2-Konzentrationen auf Grund der fehlenden reifenden Follikel in einem wesentlich niedrigerem Bereich, so dass die Postmenopause folglich durch ein androgeneres Verhältnis von Estrogenen zu Androgenen gekennzeichnet ist (1).

In den folgenden vier Unterkapiteln werden die in früheren epidemiologischen Beobachtungsstudien erfassten Effekte niedriger bzw. erhöhter Sexualhormonkonzentrationen/SHBG in Bezug auf kardiovaskuläre Risikofaktoren und Endpunkte bei Frauen dargestellt.

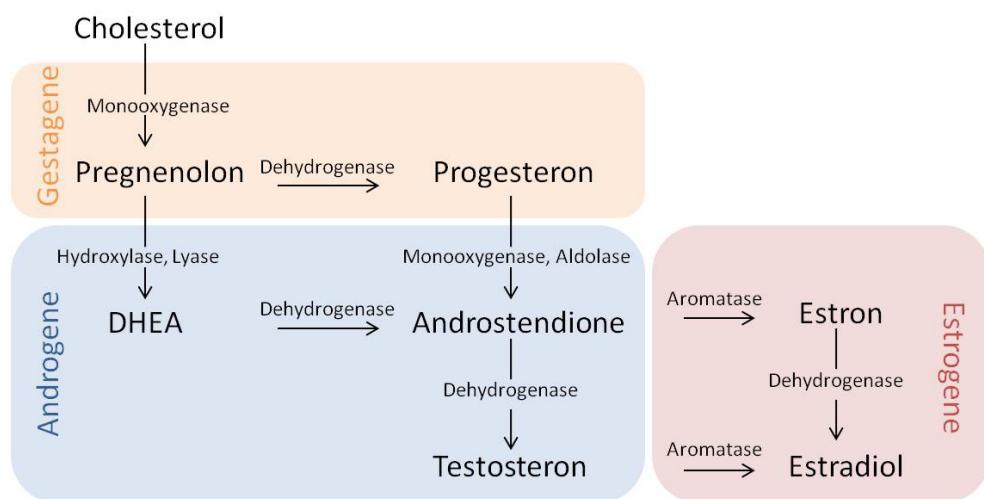


Abbildung 1: Biosynthesewege ausgewählter Sexualhormone.
DHEA, Dehydroepiandrosteron.

1.2 Sexualhormone und deren klinische Korrelate

Neben der Vielzahl an Studien, die den Zusammenhang von niedrigen T-Konzentrationen bei Männern mit der Entwicklung eines Metabolischen Syndroms (MetS) (8), Typ 2 Diabetes mellitus (T2DM) (9), Hypertonie (10) und einer Dyslipidämie (11) beschreiben, befassen sich in den letzten Jahren deutlich mehr Studien auch mit weiblichen Studienpopulationen. In einer Reihe epidemiologischer, klinischer und experimenteller Arbeiten konnte bei Frauen neben der Altersabhängigkeit der Sexualhormone/SHBG (7) ein Einfluss von Sexualhormonen auf Körpergewicht und Fettverteilung, Lipidstoffwechsel, Vasomotorik, Gerinnungs- und Fibrinolyseaktivität, aber auch auf metabolische Veränderungen, immunologische Vorgänge und das zentrale Nervensystem nachgewiesen werden (2, 6, 12, 13). Doch im Gegensatz zu den bisher gezeigten Zusammenhängen bei Männern, wurden bei Frauen erhöhte Testosteron-

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bzw. geringe SHBG-Konzentrationen mit einer gesteigerten kardiovaskulären Risikofaktorbelastung korreliert (14-16) und Zusammenhänge zwischen klinischen Korrelaten und Sexualhormonen/SHBG bei Frauen proklamiert. So war bei Frauen beispielsweise ein steigender Body Mass Index (BMI) mit niedrigen SHBG- und hohen E2-Konzentrationen assoziiert (6, 17). Zudem zeigten Sexualhormone wie E2 einen ausgeprägten Effekt auf die Fettspeicherung und das Körpergewicht (18). Des Weiteren wurde ein Zusammenhang zwischen systolischem Blutdruck bzw. Bluthochdruck und freiem Testosteron (fT) und dem Gesamt-Testosteron (T) beschrieben (19). Im Hinblick auf die Hormonveränderungen in der Menopause konnte darüber hinaus gezeigt werden, dass prämenopausale im Vergleich zu postmenopausalen Frauen sowohl niedrigere Low-density Lipoprotein (LDL)-Cholesterol als auch höhere High-density Lipoprotein (HDL)-Cholesterolkonzentrationen aufwiesen (20). Zusammenfassend lassen diese vorausgegangene Studien demnach die Hypothese zu, dass verschiedene kardiovaskuläre Risikofaktoren mit einzelnen Sexualhormonen oder SHBG korrelieren.

1.3 Sexualhormone und das Metabolische Syndrom und Typ 2 Diabetes mellitus

Schätzungsweise 256 Millionen Patienten im Alter zwischen 20-79 Jahren leiden weltweit unter T2DM (21). In Deutschland wird die Zahl der an T2DM Betroffenen für das Jahr 2030 auf 7,2 Millionen geschätzt (22). Patienten mit T2DM haben ein erhöhtes Risiko für kardiovaskuläre Krankheiten (KVK), wobei dieses Risiko bei Frauen deutlich höher beschrieben wurde als bei Männern (23). Auch für das MetS, welches bei 20-25% der weltweiten volljährigen Bevölkerung festgestellt werden kann, wird eine steigende Inzidenz beobachtet (24). Das MetS wird als das gemeinsame Auftreten von mindestens drei festgelegten, multiplen, metabolischen Veränderungen definiert, welche die kardiovaskulären Risikofaktoren viszerale Fettleibigkeit, erhöhter Blutzucker, erhöhte Triglyceride, erniedrigtes HDL-Cholesterol sowie erhöhter Blutdruck einschließen (25). In früheren Beobachtungsstudien (26) und Meta-Analysen (27) konnte gezeigt werden, dass bei Frauen hohe T-Konzentrationen sowie niedrige SHBG-Konzentrationen mit dem MetS assoziiert sind. Eine weitere Studie wies einen Zusammenhang zwischen SHBG und den einzelnen MetS-Komponenten bei postmenopausalen Frauen nach (28). Unabhängig von der Altersabhängigkeit des T2DM deutet eine Vielzahl an epidemiologischen Studien darauf hin, dass ein Zusammenhang zwischen Androgenen/SHBG und T2DM besteht (29). Zudem wurde in einer Studie mit postmenopausalen Frauen festgestellt, dass bei dem Zusammenhang zwischen hohen T- Konzentrationen und steigendem T2DM Risiko eine Abhängigkeit von

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Fettleibigkeit, Insulinresistenz oder SHBG besteht (30, 31). Zusammenfassend waren demnach hohe T- und niedrige SHBG-Konzentrationen bei Frauen sowohl mit MetS als auch mit T2DM assoziiert, auch wenn diese Zusammenhänge nicht gänzlich unabhängig von Kofaktoren bestanden.

1.4 Sexualhormone und subklinische kardiovaskuläre Krankheiten

Als Parameter für subklinische KVK können Anzeichen für die subklinische Atherosklerose der Halsgefäße herangezogen werden, die durch die Intima Media Dicke (IMD) der Arteria carotis communis und die Bildung von Carotisplaques repräsentiert werden. Letztere entstehen durch die Veränderung in den Mesenchymzellen der Gefäßwände (32). Auch das kardiale Remodeling ist in seiner leichten Ausprägung als subklinischer KVK zu betrachten (33). Dieser kardiale Umbauprozess entsteht bei großer Belastung des Herzens und beginnt mit einer initialen, adaptiven Anpassungsreaktion. Im weiteren Verlauf kann es zu einem progredienten Remodeling kommen, das zu einer Verschlechterung der Herzfunktion führt. Das kardiale Remodeling kann durch Veränderungen in der linksventrikulären Masse, Wanddicke und Geometrie beschrieben werden. In Tiermodellen und epidemiologischen Beobachtungsstudien, mit allerdings teils diametralen Resultaten, konnten Zusammenhänge zwischen Sexualhormonen/SHBG mit subklinischen kardiovaskulären Parametern gefunden werden (32, 34-36). So wurden Assoziationen zwischen T, fT und SHBG und IMD bei Frauen nachgewiesen (32, 37, 38). Hingegen blieben in einer anderen Studie, bei der Frauen während des Klimakteriums untersucht wurden, signifikante Zusammenhänge zwischen T-Konzentrationen und IMD aus (39). Des Weiteren konnte eine Studie zum SHBG-Genpolymorphismus den Zusammenhang zwischen SHBG und Atherosklerose bestätigen, indem sie eine Assoziation zwischen dem SHBG-Genpromotor-Polymorphismus und Markern der Atherosklerose nachwies (40). Beobachtete Geschlechtsunterschiede bei der Entstehung und im Verlauf des kardialen Remodelings unterstützen die Hypothese, dass Sexualhormone außerdem geschlechtsspezifische Einflüsse auf subklinische KVK haben (41). In der Gesamtheit der Studien wurden demnach Zusammenhänge zwischen Androgenen und subklinischen kardiovaskulären Erkrankungen bei Frauen nachgewiesen, deren Signifikanz allerdings stark von der ausgewählten Studienpopulation abhing.

1.5 Sexualhormone und klinische kardiovaskuläre Krankheiten und Mortalität

Wie auch in den Vorjahren weist die aktuelle Todesursachenstatistik 2014 Erkrankungen des Herz-Kreislaufsystems als die häufigste Todesursache bei Frauen aus. In Deutschland

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übertrifft dabei der Anteil der kardiovaskulären Mortalität der Frau mit 56,1% den Anteil der Männer (43,9%) deutlich (42). Hierbei lässt sich ein postmenopausaler Anstieg von KVK beobachten, der mit sehr geringen Raten in der Prämenopause und einem erheblichen Anstieg des Risikos ab dem 50. Lebensjahr und steilen Anstieg nach dem 60. Lebensjahr einhergeht (42). Als ursächlich für diese Erkrankungsmuster wurden hormonelle und stoffwechselbezogene Erklärungsansätze proklamiert. Studien haben gezeigt, dass sehr niedrige und sehr hohe T-Konzentrationen einen Risikomarker für KVK und KVK-Mortalität bei Männern darstellen (43, 44) und dass T als potentieller Biomarker für die Ausprägung von KVK Risikofaktoren in Betracht kommt (35). Bei Frauen ist dieser Zusammenhang weniger gut untersucht, doch es gibt Hinweise darauf, dass das Fehlen des putativen kardioprotektiven Estrogen- und Progesteroneinflusses während des relativ langen postmenopausalen Lebensabschnittes eine mögliche Ursache des erhöhten postmenopausalen kardiovaskulären Risikos sein könnte. Auch Androgen- und SHBG-Konzentrationen verändern sich während der Menopause und es wurde beobachtet, dass hohe T- und niedrige SHBG-Konzentrationen bei Frauen für ein höheres KVK-Risiko (26, 45) und für eine höhere Inzidenzrate kardiovaskulärer Morbidität sprechen (26, 46). Ein ähnliches Bild zeichnete eine weitere Beobachtungsstudie, in der niedrige fT-Konzentrationen bei postmenopausalen diabetischen Frauen sowohl mit kardiovaskulärer als auch mit der Gesamt-Mortalität assoziiert waren (47).

1.6 Ziel der Arbeit

Eine wesentliche Limitation der meisten oben genannten epidemiologischen Beobachtungsstudien stellt die Sexualhormonmessung mittels Immunoassays dar, die für den diagnostisch relevanten, niedrigen Messbereich der Androgene bei Frauen eine nur unzureichende Sensitivität und analytische Qualität bietet. In der vorliegenden Arbeit hingegen wurden für die Androgene T und AD massenspektroskopische Verfahren eingesetzt, die im Vergleich erheblich geringere Intra- und Interlaborvariabilität aufweisen (48, 49). Weitere Limitationen bisheriger Studien sind die teilweise stark selektierten oder kleinen Studienpopulationen oder ein ausschließliches Querschnittsdesign. Zusammenfassend lassen die bisherigen Beobachtungsstudien die Hypothese zu, dass erhöhte bzw. niedrige Sexualhormon- und SHBG-Konzentrationen aussagekräftige Biomarker des kardiovaskulären Risikos der Frau sein könnten (Abbildung 2) (50). Ob allerdings der postmenopausale Hormonstatus *per se* als Risikofaktor für KVK und KVK-Mortalität gesehen werden kann - insbesondere über seinen negativen Einfluss auf Blutdruck, Fettstoffwechsel, Insulinresistenz und abdominaler Fettleibigkeit hinaus - ist bislang noch ungeklärt (51).

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Ziel der vorliegenden Arbeit war es, den Zusammenhang zwischen Sexualhormonen/SHBG bei Frauen und einem breiten Spektrum kardiovaskulärer Risikofaktoren einschließlich klinischer Korrelate, MetS, T2DM und subklinischer KVK, sowie klinisch manifestierter KVK und Mortalität zu untersuchen. In Hinblick auf die Limitationen der genannten epidemiologischen Beobachtungsstudien wurden diese Zusammenhänge in einer populationsbasierten Stichprobe von rund 2000 Frauen im Alter zwischen 20 und 79 Jahren mit massenspektrometrischer Sexualhormonbestimmung und Fünf- bzw. Zehn-Jahres Follow-up analysiert. Zusätzlich galt es zu ermitteln, ob etwaige Zusammenhänge bei prä- oder postmenopausalen Frauen unterschiedlich ausfallen und unabhängig von Verhaltensrisikofaktoren, Komorbiditäten, Hormontherapie (HT) oder Fettleibigkeit auftreten.

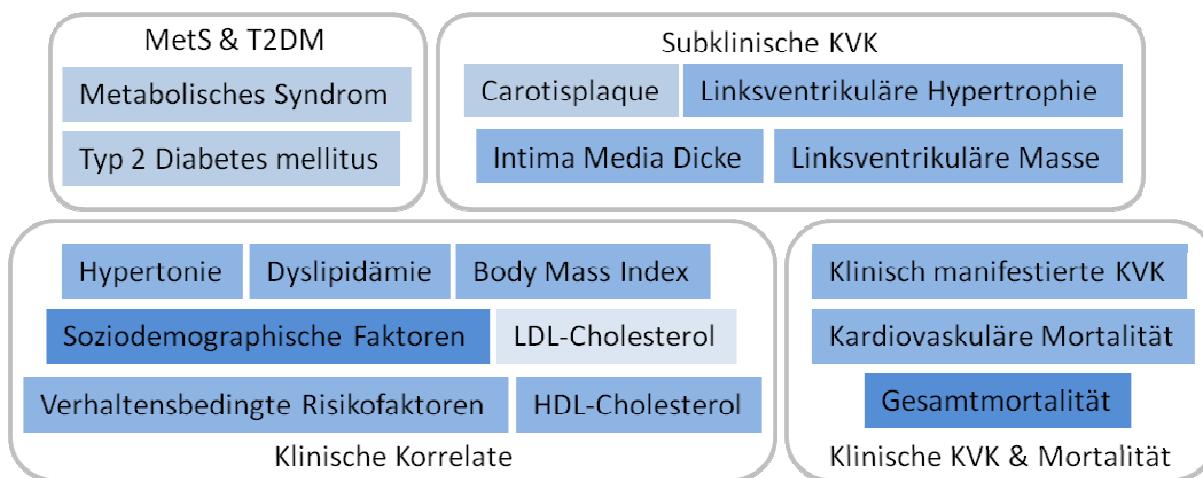


Abbildung 2: Überblick der Zusammenhänge zwischen Sexualhormonen/Sexualhormon-bindendem Globulin und kardiovaskulären Risikofaktoren/Mortalität bei Frauen. Ausgehend vom derzeitigen Forschungsstand wurden die Faktoren umso dunkler eingefärbt, je stärker sie mit den Sexualhormonen und desto weniger stark sie mit SHBG assoziiert sind (und *vice versa*).

MetS, Metabolisches Syndrom, T2DM, Typ 2 Diabetes mellitus; KVK, kardiovaskuläre Krankheiten, LDL, low-density Lipoprotein; HDL, high-density Lipoprotein.

2. Methoden

2.1 Studiendesign und Studienpopulation

Die Daten, die dieser Arbeit zugrunde liegen, stammen aus der populationsbasierten, longitudinalen Study of Health in Pomerania (SHIP). Ein Überblick über die Durchgänge der SHIP ist in Tabelle 1 dargestellt. Es wird angestrebt, sowohl Prävalenzen, Inzidenzen und Risikofaktoren eines weiten Spektrums subklinischer und klinisch manifestierter Krankheiten in Nordostvorpommern, als auch deren Zusammenhänge zu ermitteln. Mittels eines zweistufigen Prozesses wurde im ersten Schritt eine randomisierte, repräsentative Stichprobe der 20-79-jährigen Einwohner gezogen. Einbezogen wurden Einwohner mit deutscher Staatsangehörigkeit und Hauptwohnsitz in den Landkreisen Ost- und Nordvorpommern sowie den kreisfreien Städten Greifswald und Stralsund. Im zweiten Ziehungsdurchgang wurde eine randomisierte, alters- und geschlechtsstratifizierte Ziehung der Probanden aus den Registern der Einwohnermeldeämter der jeweiligen Städte durchgeführt. Der Umfang des Untersuchungsprogrammes umfasst unter anderem in einer Biobank asservierte Plasma-, Urin-, Speichel- und Serumproben, ein computerassistiertes, persönliches Interview mit Informationen zu sozioökonomischen Faktoren und Risikoverhalten und außerdem medizinische Untersuchungen einschließlich Blutdruckmessung, Elektrokardiogramm, Schlaflabor, somatometrische Messungen und Sonographien. Medikamente, die von den Probanden in den vorausgegangenen sieben Tagen eingenommen wurden, wurde mittels der amtlichen deutschen Fassung der anatomisch-therapeutisch-chemischen Klassifikation (ATC) der Wirkstoffname zugeordnet. Über weitere Details zur Studienpopulation, -region und -design wird an anderer Stelle berichtet (52, 53). Ausgeschlossen wurden für alle Analysen in der vorliegenden Arbeit: Männer, schwangere Frauen, Frauen mit bilateraler Oophorektomie oder Hysterektomie und Frauen, die den Hormonhaushalt beeinflussende Medikamente einnahmen.

Tabelle 1: Überblick über die Durchgänge der Study of Health in Pomerania.

	N	Response	N Frauen	Zeitraum der Untersuchungen
SHIP-0 Basisuntersuchung erste Kohorte	4308	68,8%	2192	1997-2001
SHIP-1 Fünf-Jahres Follow-up erste Kohorte	3300	83,6%	1711	2002-2006
SHIP-2 Zehn-Jahres Follow-up erste Kohorte	2333	54,2%	1235	2008-2012
SHIP-TREND Basisuntersuchung zweite Kohorte	4420	50,1%	2275	2008-2012

SHIP, Study of Health in Pomerania.

2.2 Erfassung und Definition von metabolischen und endokrinen Variablen

Alle Assays wurden durch ausgebildetes, technisches Personal und nach den Empfehlungen des Herstellers durchgeführt.

2.2.1 Labormethodik zur Bestimmung der Sexualhormone und SHBG

Die Serumproben wurden mittels Blutabnahme aus der Kubitalvene der überwiegend nicht nüchternen Probanden gewonnen und bei -80 °C gelagert. Für die Messungen von T und AD in beiden SHIP Basisuntersuchungen und E1 und E2 in SHIP-TREND wurden die Aliquots in das Department of Clinical Chemistry des University Hospital of South Manchester (Manchester, UK) überführt. In diesem wurden Messungen der Sexualhormone mittels validierter Flüssigkeitschromatographie/ Tandem-Massenspektrometrie durchgeführt (54). Die Standardkurve war linear zu 50,0 nmol/L und die untere Bestimmungsgrenze des Assays betrug 0,25 nmol/L. Der Interassay-Variationskoeffizient für T und AD im Bereich von 0,3 bis 35 nmol/L lag bei <10%. Der Messbereich für die Estrogene E1 und E2 betrug 25–2000 pmol/L. Das untere Detektionslimit befand sich für E1 bei 3,9 pmol/L und für E2 bei 8,0 pmol/L (55). In SHIP-0 wurde SHBG mittels Radioimmunoassay (Advia Centaur, Siemens, Eschborn, Deutschland) mit einem Interassay-Variationskoeffizienten von 6,6% bei 27,1 nmol/L, 7,6% bei 48,2 nmol/L und 7,7% bei 52,3 nmol/L gemessen. In SHIP-TREND wurden SHBG sowie Dehydroepiandrosteron-Sulfat (DHEAS) mittels kompetitivem Chemilumineszenz-Immunoassay (Immulite 2000 XPi, Siemens Healthcare Diagnostics GmbH, Eschborn, Deutschland) gemessen. FT (Formel A1-4) und der Freie Androgenindex (FAI) (Formel B) wurden mit den folgenden Formeln aus T und SHBG berechnet (56):

$$fT \text{ [nmol/L]} = ((-a + \sqrt{b})/c) / 10^{-9} \quad (\text{A1})$$

$$a = SHBG \text{ [nmol/L]} - T \text{ [nmol/L]} + 23,43 \quad (\text{A2})$$

$$b = a^2 + (4 \cdot 23,43 \cdot T \text{ [nmol/L]}) \quad (\text{A3})$$

$$c = 2 \cdot 23,43 \cdot 10^9 \quad (\text{A4})$$

$$FAI = T \cdot 100 / SHBG \quad (\text{B})$$

2.2.2 Klinische Korrelate

Sämtliche in dieser Arbeit analysierten klinischen Korrelate wurden sowohl in SHIP-0 als auch in SHIP-TREND erfasst. Im Rahmen der anthropometrischen Messungen wurden Größe und Taillenumfang (Präzision: 0,1 cm), sowie das Gewicht (Präzision: 0,1 kg) der Probanden

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mit geeichten Geräten gemessen und der BMI aus dem Gewicht in kg und der Körpergröße in m ($BMI = \text{Gewicht}/\text{Körpergröße}^2$) berechnet. Der Taillenumfang wurde mit einem unelastischen Maßband an der schmalsten Stelle zwischen der letzten Rippe und der höchsten Stelle des Darmbeinkammes bestimmt. Der mittlere Tagesalkoholkonsum wurde aus dem Anteil des reinen Ethanolvolumens eines jeden zugeführten alkoholischen Getränktes berechnet. In Bezug auf physische Inaktivität wurden Frauen, die weniger als eine Stunde Sport pro Woche trieben als physisch inaktiv eingestuft. Hinsichtlich des Raucherstatus wurden die Probanden basierend auf dem persönlichen Interview in die folgenden drei Kategorien eingeteilt: Nichtraucher, früherer Raucher oder derzeitiger Raucher.

Folgende soziodemographische Variablen wurden aus dem persönlichen Interview und dem Fragebogen zum Selbstausfüllen gewonnen und teilweise in weitere Kategorien eingeteilt:

- Schulbildung: <10, 10 oder >10 Schuljahre
- Monatliches Netto-Haushaltseinkommen: <1000 €, 1000-1500 € oder >1500 €
- Subjektive Gesundheit: ausgezeichnet, sehr gut, gut, weniger gut oder schlecht
- Kohabitation: alleinstehend, verheiratet oder mit Partner lebend, getrennt lebend oder geschieden, verwitwet

Des Weiteren wurde der systolische und diastolische Blutdruck dreimal am rechten Arm der sitzenden Probanden ermittelt, nachdem sie eine Ruhephase von mindestens fünf Minuten eingehalten hatten. Die Messungen erfolgten im Abstand von jeweils drei Minuten unter Verwendung eines automatischen, oszillometrischen Messgeräts (HEM-705CP, OMRON Corporation, Tokyo, Japan). Der Mittelwert aus zweiter und dritter Messung wurde für alle statistischen Analysen verwendet. Ein Bluthochdruck wurde bei Probanden mit einem Blutdruck $\geq 140/90$ mmHg und/oder mit der Einnahme antihypertensiver Medikamente definiert (ATC Codes C02, C03, C04, C07, C08, C09) (57). Triglyceride und Glukose wurden im Serum mit enzymatischen Methoden gemessen (Hitachi 717, Roche Diagnostics GmbH, Mannheim, Germany). Das HDL-Cholesterin wurde in SHIP-0 photometrisch mittels eines Hitachi 704, Roche Diagnostics, (Mannheim, Deutschland) erhoben und in SHIP-TREND mittels eines Dimension Vista 500 analytical systems.

Dyslipidämie und Krebserkrankungen wurden auf Grundlage der Selbstangabe ärztlicher Diagnose erfasst. Auf die Definitionen des MetS und des T2DM, die ebenfalls in die Analysen der klinischen Korrelate eingeschlossen wurden, wird im folgenden Unterkapitel (2.2.3) eingegangen.

2. Methoden

2.2.3 Definitionen des Metabolischen Syndroms und des Typ 2 Diabetes mellitus

Die Definitionen für MetS und für T2DM wurden sowohl in SHIP-0 und -1 als auch in SHIP-TREND formuliert. Das MetS wurde auf Grundlage des „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“ definiert (58). Dementsprechend beinhaltet die Definition des MetS fünf einzelne Komponenten, die in der vorliegenden Arbeit als bivariate Variablen kodiert wurden:

- Taillenumfang ≥ 102 cm
- Blutdruck $\geq 130/85$ mmHg und/oder Selbstangabe antihypertensiver Medikation
- nicht nüchterne Glukosekonzentration $\geq 6,1$ mmol/L oder Selbstangabe antidiabetischer Medikation (ATC Codes A10A, A10B)
- nicht nüchterne Triglyceridkonzentration $\geq 1,7$ mmol/L oder lipidsenkende Medikation (ATC Codes C10AB, A10AD)
- HDL-Cholesterol $\leq 1,3$ mmol/L.

Ein MetS wurde festgestellt, wenn ein Proband mindestens drei dieser Komponenten erfüllte. Da die Blutproben in SHIP-Core von überwiegend nicht nüchternen Probanden stammten, wurden die Schwellenwerte für Glukose und Triglyceride an nicht nüchterne Proben angepasst (59).

Hinsichtlich des T2DM wurden Probanden mit selbstangegebener ärztlicher Diagnose des T2DM, mit Einnahme von antidiabetischen Medikamenten (ATC Code A10) und/oder mit glykosyliertem Hämoglobinlevel ($\text{HbA1c} \geq 6,5\%$ und $< 20\%$) als diabetisch definiert.

2.2.4 Subklinische kardiovaskuläre Krankheiten

Es wurden Daten von folgenden sieben subklinischen Variablen aus SHIP-0 und -1 in die Analysen eingeschlossen. Die mittlere IMD der Arteria carotis communis wurde definiert als der Mittelwert aus zehn aufeinanderfolgenden, sonographisch ermittelten Distanzen zwischen den Übergangsstellen von Lumen-Tunica intima und Tunica media-Tunica adventitia der beiden Arteria carotis communes. Ebenfalls mittels Sonographie der Arteria carotis communis wurde das Vorhandensein von Carotisplaques festgestellt. Die Echokardiographie des linken Ventrikels einschließlich posteriorer Wanddicke (LVPWD), interventrikulärer Septumdicke ((IVS) und linksventrikulärer end-diastolischer (LVDD) und systolischer (LVDS) Diameter, stellte die Basis zur Berechnung der weiteren subklinischen Parameter dar:

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- Linksventrikulärer Massenindex =
$$0,80 \times (1,04 \times [[\text{LVDD} + \text{IVS} + \text{LVPWD}]^3 - \text{LVDD}^3]) + 0,60 / \text{Körpergröße}^{2,7}$$
- Linksventrikuläre Hypertrophie (LVH) = $\text{LVM} > 44 \text{ g/m}^{2,7}$ (60)
- Relative Wanddicke (RWD) = $(2 \times \text{LVPWD}) / \text{LVDD}$
- Verkürzungsfaktion = $([\text{LVDD} - \text{LVDS}] / \text{LVDD}) \times 100$
- Linksventrikuläre Geometrie
 - normale Geometrie: keine LVH und RWD $< 0,42$
 - konzentrische Geometrie: keine LVH und RWD $> 0,42$
 - exzentrische Geometrie: LVH und RWD $< 0,42$
 - konzentrische Hypertrophie: LVH und RWD $> 0,42$ (61)

2.2.5 Klinische kardiovaskuläre Krankheiten und Mortalität

KVK wurden als binäre Variable (ja/nein) in SHIP-0 und im Fünf- sowie im Zehn-Jahres Follow-up definiert, basierend auf fünf Items einschließlich Selbstangabe von Symptomen der Angina pectoris, peripherer arterieller Erkrankungen oder Herzfehler und selbstangegebener ärztlicher Diagnose von Schlaganfall und Herzinfarkt. Die positive Beantwortung mindestens eines der Items wurde als Kriterium für eine bereits bestehende KVK gewertet.

Die kardiovaskuläre Mortalität wurde mittels Kodierung der Sterbeurkunden nach der Internationalen statistischen Klassifikation der Krankheiten und verwandter Gesundheitsprobleme (ICD-10) der Weltgesundheitsorganisation von unabhängigen nosologisch ausgebildeten, zertifizierten Medizinern erfasst. Anschließend wurde die zugrundeliegende Todesursache von zwei unabhängigen Internisten validiert und bei Unstimmigkeiten ein Dritter hinzugezogen.

2.2.6 Zusätzlich berücksichtigte Kovariaten

Zur Erstellung der multivariablen Modelle wurden folgende zusätzliche Einflussgrößen herangezogen: Alter in Jahren und Zehn-Jahresgruppen, Verwendung von oralen Kontrazeptiva (ATC Code G03A) und HT (ATC Code G03C, G03D oder G03F), Zeitpunkt der Blutabnahme, Einnahme von Medikamenten (ja/nein) und Menopause (prä- oder postmenopausal). Als prämenopausal wurden Frauen < 40 Jahre und Frauen zwischen 40-60 Jahren mit selbstangegebenem Menstruationszyklus definiert. Entsprechend wurden Frauen ≥ 60 Jahre und Frauen zwischen 40-60 Jahren, die einen fehlenden Menstruationszyklus berichteten als postmenopausal definiert (62).

2.3 Statistische Methoden

Um die Stichprobe zu beschreiben, wurden Verfahren der deskriptiven Statistik angewandt. Gruppenunterschiede wurden mit dem Chi-Quadrat Test bei nominalskalierten und mit dem Kruskal-Wallis Test bei kontinuierlichen Variablen auf statistische Signifikanz getestet. Um eine Normalverteilung der Variablen zu erreichen, wurden grundsätzlich die Sexualhormone und bei Schiefe ebenfalls abhängige Variablen transformiert oder kategorisiert, wenn dies eine Voraussetzung für das entsprechende Regressionsmodell war. Zur Erfassung der Zusammenhänge zwischen Sexualhormonen/SHBG mit klinischen Korrelaten und Erkrankungen wurden in Tabelle 2 ersichtliche Regressionsmodelle formuliert. Alle Effekte wurden mit deren 95% Konfidenzintervallen berechnet. Linearen Regressionen folgte eine Prüfung der Regressionsresiduen mittels QQ-Plots. Hinsichtlich der Überlebenszeiten wurden Kaplan-Meier Graphen dargestellt und die Überlebenskurven mit Hilfe des Log-rank Tests verglichen. Die Annahme der proportionalen Hazards wurde auf Basis der Schoenfeld Residuen überprüft.

Um in multivariablen Modellen Autokorrelation zu vermeiden, wurde stets das kleinstmögliche Kofaktorenset gewählt. Jede multivariable Analyse enthielt die Kofaktoren Alter, BMI oder Tailenumfang, Raucherstatus, physische Inaktivität und Alkoholkonsum und wurde zusätzlich durch modellspezifische Kofaktoren ergänzt. Anschließend wurden, basierend auf signifikanten multiplikativen Interaktionstermen bzw. hypothesenbasiert auf Grundlage früherer Studien, Sensitivitätsanalysen durchgeführt. Hierbei wurden die Regressionsmodelle für die Menopause und für die Verwendung von oralen Kontrazeptiva und HT stratifiziert. Um repräsentative Aussagen für die Studienregion treffen zu können, wurden alle Analysen basierend auf soziodemographischen und gesundheitsbezogenen Variablen für ein Ausscheiden zwischen Basis- und Follow-up Untersuchungen gewichtet. Ein p-Wert <0,05 wurde als statistisch signifikant angesehen. Alle statistischen Analysen erfolgten mit dem Programm Stata 13.0 (Stata Corporation, College Station, TX, USA).

Tabelle 2: Regressionsmodelle, separiert nach den abhängigen Variablen.

Abhängige Variable	Unabhängige Variable	Regressionsmodelle	Darstellung des Effekts
Sexualhormone/SHBG	Klinische Korrelate	Lineare Regression	β-Koeffizienten
MetS und T2DM	Sexualhormone/SHBG	Poisson Regression	Relatives Risiko
Subklinische Parameter	Sexualhormone/ SHBG	Lineare Regression Poisson Regression Logistische Regression	β-Koeffizienten Relatives Risiko Odds Ratio
KVK Mortalität	Sexualhormone/SHBG	Poisson Regression Cox-Regression	Relatives Risiko Hazard Ratio

SHBG, Sexualhormon-bindendes Globulin; MetS, metabolisches Syndrom; T2DM, Typ 2 Diabetes mellitus; KVK, Kardiovaskuläre Krankheiten.

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Ein Überblick über die Ergebnisse der vorliegenden Arbeit findet sich in folgender Tabelle.

Tabelle 3: Überblick zu den signifikanten Ergebnissen der multivariablen Regressionsmodelle.

	T	fT	AD	DHEAS	E1	E2	SHBG	FAI
KLINISCHE KORRELATE								
Body Mass Index	+	+			+	-	-	
Taillenumfang	+	+					-	
Systolischer Blutdruck	+	+	+		+			
Diastolischer Blutdruck	+	+			+		-	
Hypertonie	+	+	+					
HDL-Cholesterol	+	-					+	
LDL-Cholesterol	+	+	+	+	-		-	
Gesamt-Cholesterol	+	+	+	+	-		-	
Dyslipidämie			-				-	
Raucherstatus	+	+	+					
Physische Inaktivität	-	-	-					
METS & T2DM								
Metabolisches Syndrom								
Querschnitt		+					-	+
Längsschnitt							-	
Typ 2 Diabetes mellitus								
Querschnitt		+					-	+
Längsschnitt								
SUBKLINISCHE KVK								
Intima Media Dicke								
Querschnitt		+						
Längsschnitt								
Verkürzungsfaktion								
Querschnitt		+	+					
Längsschnitt								
Carotisplaques								
Querschnitt							-	
Längsschnitt								
KLINISCHE KVK & MORTALITÄT								
Klinische KVK								
Querschnitt								
Längsschnitt								
KVK-Mortalität								
Gesamtmortalität								

+ positive Assoziation. – inverse Assoziation. Freies Feld: keine signifikante Assoziation.

orange hinterlegt: Assoziation wurde auf Grund fehlender Daten nicht geprüft.

HDL, High-density Lipoprotein; LDL, Low-density Lipoprotein; MetS, Metabolisches Syndrom; T2DM, Typ 2 Diabetes mellitus; KVK, Kardiovaskuläre Krankheiten; T, Gesamt-Testosteron; fT, Freies Testosteron; AD, Androstendion; DHEAS, Dehydroepiandrosteron; E1, Estron; E2, Estradiol; SHBG, Sexualhormon-bindendes Globulin; FAI, Freier Androgenindex.

3.1 Sexualhormone und klinische Korrelate

Um den Zusammenhang zwischen klinischen Korrelaten und Sexualhormonen/SHBG zu untersuchen, wurden Daten von 2560 Frauen mit einem Durchschnittsalter von 49,3 Jahren aus SHIP-TREND herangezogen. Es wurde beobachtet, dass die Sexualhormone/SHBG eine altersabhängige Verteilung zeigen. Bezuglich somatometrischer Daten stellte sich ein signifikanter Zusammenhang zwischen BMI und E1, E2, T, fT und SHBG im

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altersadjustierten Modell heraus (Abbildung 3 A1 und A2). Der Taillenumfang war auch in multivariablen Modellen invers mit SHBG assoziiert. Hinsichtlich soziodemographischer Daten konnten Zusammenhänge zwischen Raucherstatus und T, fT und AD gefunden werden. Es zeigte sich ein inverser Zusammenhang zwischen physischer Inaktivität sowie Alkoholkonsum und fT. Außerdem wurde ein positiver Zusammenhang zwischen Netto-Haushaltseinkommen und E2 sowie zwischen Kohabitation und E1, E2, T und DHEAS beobachtet. In Bezug auf kardiometabolische Variablen waren der systolische Blutdruck (Abbildung 3 B1 und B2), Bluthochdruck, LDL-Cholesterol und das Gesamt-Cholesterol positiv mit T und AD und invers mit SHBG assoziiert. Dementsprechend zeigte LDL-Cholesterol einen inversen Zusammenhang mit SHBG. In der Hauptanalyse wurde ein Zusammenhang zwischen Dyslipidämie und E2 detektiert, während im Rahmen einer Sensitivitätsanalyse dieser Zusammenhang bei prämenopausalen Frauen auch für T, fT und DHEAS festgestellt wurde.

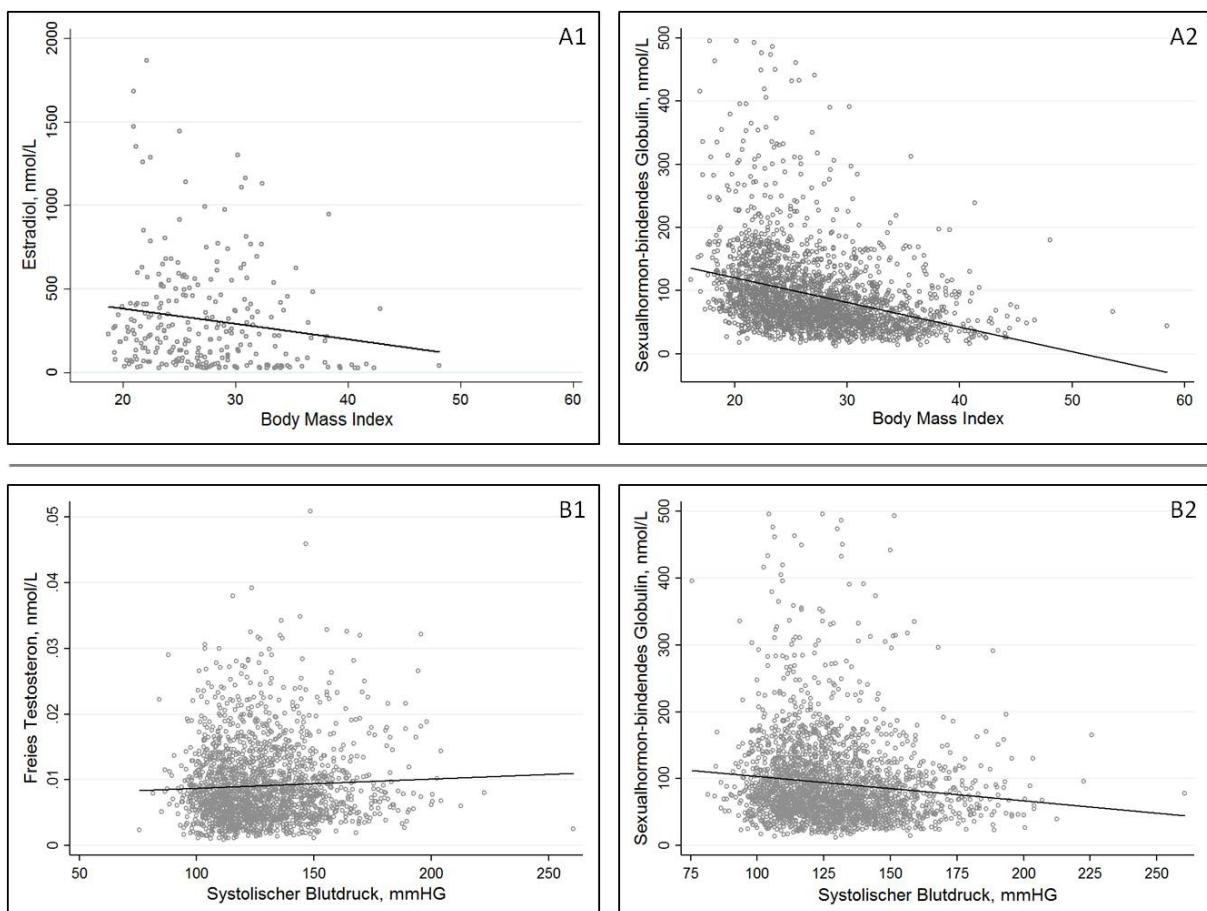


Abbildung 3: Streudiagramm für den Zusammenhang zwischen ausgewählten klinischen Korrelaten und Sexualhormonen/Sexualhormon-bindendem Globulin bei Frauen.

A1) Body Mass Index und Estradiol (N = 260), A2) Body Mass Index und Sexualhormon-bindendes Globulin (N = 2406). B1) systolischer Blutdruck und freies Testosteron (N = 1960), B2) systolischer Blutdruck und Sexualhormon-bindendes Globulin (N = 2406).

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3.2 Sexualhormone und das Metabolische Syndrom und Typ 2 Diabetes mellitus

Zur Klärung der zweiten Fragestellung nach einem Zusammenhang zwischen Sexualhormonen/SHBG und dem MetS und T2DM bei Frauen in SHIP-0 und -1 wurden 2077 Frauen mit einem Durchschnittsalter von 48,8 Jahren herangezogen. Prämenopausale Frauen wiesen im Vergleich zu postmenopausalen Frauen eine niedrigere Prävalenz für Bluthochdruck, MetS und T2DM auf.

Hinsichtlich des MetS bei Frauen lag die Prävalenzrate bei 23,1% und die Inzidenzrate bei 33,3%. Die MetS-Inzidenz pro 1000 Personenjahre betrug 17,7. Es konnten eine inverse Assoziation zwischen SHBG und MetS sowohl in multivariablen Querschnitts- als auch in Längsschnittanalysen nachgewiesen werden. Auch für fT konnte eine signifikante Assoziation mit MetS detektiert werden, allerdings war es im Gegensatz zu SHBG positiv assoziiert (Abbildung 4A) und in Längsschnittanalysen ausschließlich im altersadjustierten Modell. Für niedrige SHBG-Konzentrationen konnte ein signifikanter Trend mit steigenden MetS-Komponenten gezeigt werden.

Bezüglich des T2DM bei Frauen lag die Prävalenzrate bei 9,5% mit einer im Vergleich hierzu nur leicht erhöhten Inzidenzrate von 10,9%. Die T2DM Inzidenz pro 1000 Personenjahre betrug 7,0. Es zeigten sich ähnliche Resultate wie bei dem MetS (Abbildung 4B), jedoch konnte der Zusammenhang zwischen SHBG und T2DM in Längsschnittanalysen nicht in multivariablen Modellen nachgewiesen werden.

In den Sensitivitätsanalysen für beide Expositionsvariablen zeigten sich keine wesentlichen Veränderungen der Schätzer. In einer zusätzlichen, schrittweisen, multivariablen Regression erwies sich der Taillenumfang als der Kofaktor, der den größten Einfluss auf die Unterschiede in den altersadjustierten und multivariablen Modellen hatte.

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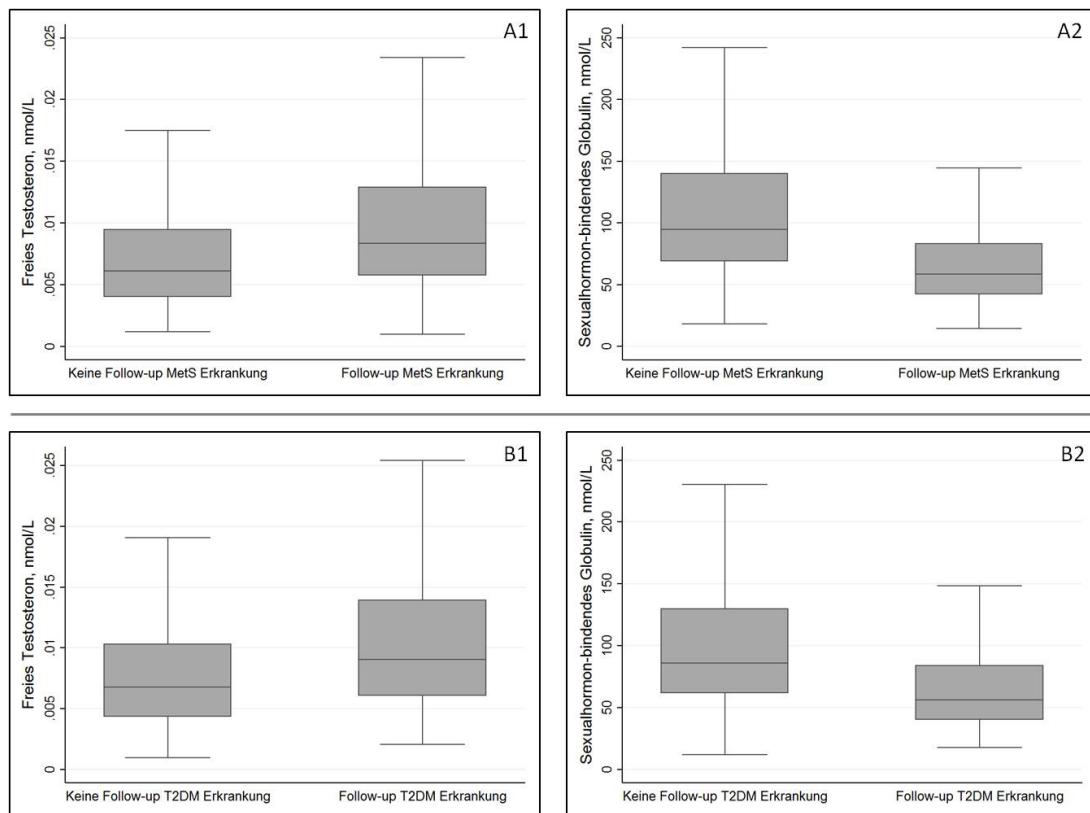


Abbildung 4: Boxplot für den Zusammenhang von freiem Testosteron und Sexualhormon-bindendem Globulin mit inzidentem Metabolischen Syndrom bzw. Typ 2 Diabetes mellitus.

A1) Freies Testosteron und Metabolisches Syndrom, A2) Sexualhormon-bindendes Globulin und Metabolisches Syndrom, B1) Freies Testosteron und Typ 2 Diabetes mellitus, B2) Sexualhormon-bindendes Globulin und Typ 2 Diabetes mellitus.

MetS, Metabolisches Syndrom; T2DM, Typ 2 Diabetes mellitus.

3.3 Sexualhormone und subklinische kardiovaskuläre Krankheiten

Der Zusammenhang zwischen Sexualhormonen/SHBG der Frau und subklinischen kardiovaskulären Parametern wurde anhand der Daten von 1145 Frauen mit einem Durchschnittsalter von 60,7 Jahren in SHIP-0 und -1 untersucht. In den multivariablen Querschnittsanalysen wurden positive signifikante Zusammenhänge zwischen AD und der Verkürzungsfraktion und zwischen T und IMD detektiert. In multivariablen Längsschnittanalysen wurden keine signifikanten Zusammenhänge zwischen Sexualhormonen/SHBG mit subklinischen Parameter gefunden.

Von den 387 Frauen, die in der Basisuntersuchung keine Carotisplaques aufwiesen, entwickelten 200 Frauen bis zum Fünf-Jahres Follow-ups Carotisplaques. SHBG zeigte einen Zusammenhang mit prävalenten Carotisplaques in multivariablen Modellen und mit inzidenten Carotisplaques allerdings ausschließlich im altersadjustierten Modell im ersten SHBG-Quintil. Bezuglich LVH entwickelten von den 460 Frauen ohne LVH in der Basisuntersuchung 124 Frauen eine LVH bis zum Fünf-Jahres Follow-up. Hinsichtlich des Zusammenhangs zwischen Sexualhormonen und LVH sowie LV-Geometrie zeigten sich

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keine signifikanten Ergebnisse in multivariablen Modellen. In den Sensitivitätsanalysen, einschließlich Stratifizieren für die Menopause und zusätzlicher Adjustierung für orale Kontrazeptiva, HT, Zeitpunkt der Blutabnahme und inverser Wahrscheinlichkeitsgewichtung, zeigten sich keine wesentlichen Veränderungen der Schätzer für alle hier untersuchten ExpositionsvARIABLEN.

3.4 Sexualhormone und klinische kardiovaskuläre Krankheiten und Mortalität

Die Daten von 2129 Frauen mit einem Durchschnittsalter von 49,0 Jahren bildeten die Grundlage für die Untersuchung des Zusammenhangs zwischen Sexualhormonen/SHBG und klinischen KVK und Mortalität. Die Prävalenzrate für KVK lag bei 17,8% und die Inzidenz betrug 50,9 pro 1000 Personenjahre über die mittlere Follow-up Zeit von 50,9 Jahren mit einer Gesamtzahl von 94 KVK-Ereignissen. Es wurde ein inverser Zusammenhang zwischen SHBG und prävalenten KVK und ein positiver Zusammenhang zwischen fT sowie FAI und prävalenten KVK beobachtet. Die beschriebenen Assoziationen wurden nur im altersadjustierten und nicht im multivariablen Modell nachgewiesen. In den Längsschnittanalysen konnte kein Zusammenhang zwischen Sexualhormonen/SHBG und inzidenten KVK detektiert werden. Es zeigten sich ebenfalls keine signifikanten Zusammenhänge zwischen KVK- oder Gesamt-Mortalität und Sexualhormonen/SHBG (Abbildung 5). In keiner der Sensitivitätsanalysen wurden wesentliche Veränderungen der Schätzer beobachtet.

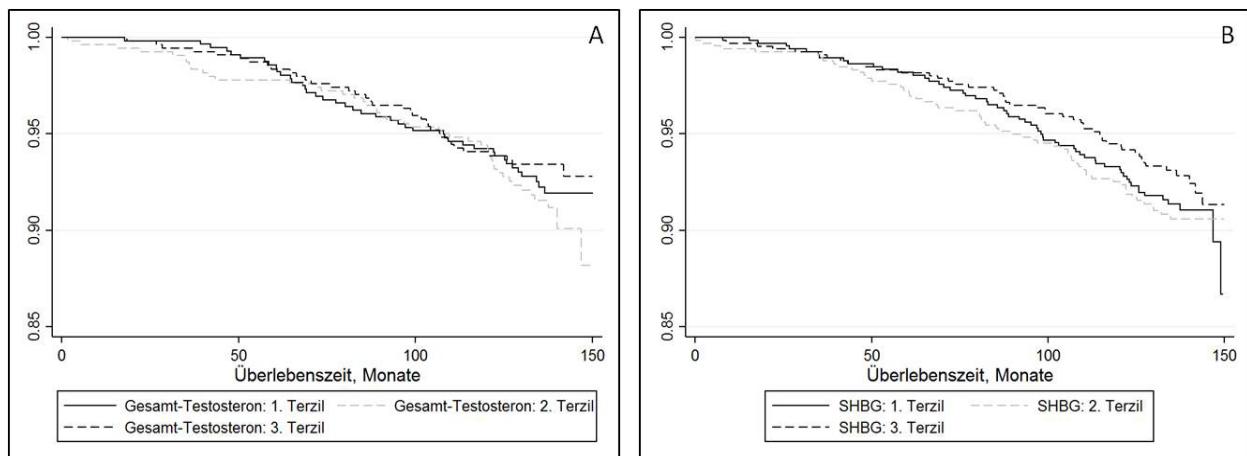


Abbildung 5: Kaplan-Meier Überlebenskurven der Gesamt-Mortalität für A) Terzile des Gesamt-Testosterons und B) Terzile des Sexualhormon-bindenden Globulins.
SHBG, Sexualhormon-bindendes Globulin.

4. Diskussion

4.1 Zusammenfassung der Ergebnisse und Einordnung in den aktuellen Forschungsstand

Die vorliegende Arbeit ist nach bestem Wissensstand die erste Studie, welche die Zusammenhänge zwischen Sexualhormonen/SHBG und einem breiten Spektrum kardiovaskulärer Risikofaktoren, KVK und Mortalität in der gesunden weiblichen Allgemeinbevölkerung in Deutschland untersucht. Wie erwartet, konnte gezeigt werden, dass alle untersuchten Sexualhormone/SHBG mit verschiedenen klinischen Korrelaten in Beziehung stehen. Außerdem konnte nachgewiesen werden, dass SHBG unabhängig von relevanten Kofaktoren mit prävalentem und inzidentem MetS assoziiert war. Hingegen wurde kein Zusammenhang zwischen Sexualhormonen/SHBG und inzidenten, subklinischen und klinischen KVK oder der Mortalität gefunden.

Hinsichtlich der klinischen Korrelate bestätigten die Ergebnisse der vorliegenden Arbeit frühere Studien (19, 63, 64) und konnten sie um den Aspekt der großen populationsbasierten, weiblichen Stichprobe erweitern. Aus der Fülle der untersuchten klinischen Korrelate soll hier deren Assoziation mit Sexualhormonen/SHBG am Beispiel des Blutdruckes und des Körpergewichtes erläutert werden. Hypertonie und systolischer Blutdruck bei Frauen war in dieser Arbeit im altersadjustierten Modell mit E1 und SHBG und im multivariablen Modell mit T und AD assoziiert. Allerdings fanden sich, besonders im Hinblick auf die Unabhängigkeit der Zusammenhänge, widersprüchliche Ergebnisse in der Literatur. Hohe E2- (65), fT- oder T-Konzentrationen (66) und niedrige SHBG-Konzentrationen (66) waren mit höherem Blutdruck assoziiert, allerdings wurden diese Assoziationen nicht in allen Studien nachgewiesen (65, 67). Näher betrachtet waren beispielsweise bei 619 postmenopausalen Frauen E2, T und DHEA positiv und SHBG invers mit dem Risiko für Hypertonie assoziiert (68). Nur für SHBG wurde eine Unabhängigkeit von Fettleibigkeit, Insulinresistenz und systematischer Inflammation beobachtet. Diese MESA-Studie zeigte, dass SHBG ein unabhängiger Prädiktor für das Neuauftreten von Hypertonie war (68), während in der vorliegenden Arbeit die Assoziationen zwischen Hypertonie/systolischem Blutdruck und SHBG sowie E1 durch den Kofaktor „BMI“ stark abgeschwächt wurden. In der Literatur wurde Fettleibigkeit sowohl als Kofaktor als auch als Mediator in der Beziehung zwischen Estrogenkonzentrationen und Hypertonie diskutiert (68).

In der vorliegenden Studie wurde ein altersunabhängiger Zusammenhang zwischen BMI und E1 sowie E2 beobachtet. Estrogene haben einen direkten Effekt auf das Fettgewebe, da sie den Metabolismus der Adipozyten regulieren. Es wird diskutiert, ob die allgemeine

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Gewichtszunahme hauptsächlich ein Effekt des Alterns und nicht der Menopause ist. Hingegen scheint die Änderung der Fettverteilung durch Hormonveränderungen tatsächlich ein Effekt der Menopause zu sein (69). Das abdominale viszerale Fett, dass nach der Menopause dreimal schneller steigt als der BMI, ist einer der Gründe für vermehrte koronare Herzerkrankungen nach der Menopause. So wiesen 456 peri- oder postmenopausale Frauen mit einem Durchschnittsalter von 50,8 Jahren ein größeres Volumen an kardiovaskulärem Fett (Fett um die Aorta und das Herz) auf, verglichen mit prä- oder frühperimenopausalen Frauen. Die Literatur zusammenfassend scheinen sowohl niedrige als auch sehr hohe endogene Estrogen-Konzentrationen ungünstige Effekte auf das kardiovaskuläre System zu haben (70). Diese Beobachtungen basieren auf der Tatsache, dass Estrogene mit verschiedenen Organsystemen im weiblichen Körper interagieren, so dass sich einzelne Auswirkungen gegebenenfalls gegenseitig überschatten (70). In den meisten Studien sind allerdings niedrige Estrogen-Konzentrationen mit einem erhöhten kardiovaskulären Risiko assoziiert, wie beispielsweise in der Copenhagen City Heart Study mit 4716 Frauen zwischen 49-65 Jahren (71). Diese Ergebnisse decken sich mit den Resultaten aus der vorliegenden Studie, in der E2-Konzentrationen invers mit kardiovaskulären Risikofaktoren, wie zum Beispiel Dyslipidämie assoziiert waren.

Prävalentes und inzidentes MetS und prävalenter T2DM waren in der vorliegenden Studie unabhängig und invers mit SHBG und positiv mit fT und FAI assoziiert. Ähnliche Ergebnisse lieferten frühere Beobachtungsstudien (26, 28, 30, 72) und Metastudien (27, 31) zu diesem Thema, in denen allerdings teilweise auch hohe T-Konzentrationen mit MetS (27) und T2DM (31) bei Frauen assoziiert waren. Diese Unterschiede in den Resultaten können größtenteils durch die verschiedenen Studienpopulationen (Größe und Durchschnittsalter der Studienpopulation, Analyse ausschließlich postmenopausaler Frauen) und Differenzen in den Messmethoden (Immunoassay vs. Massenspekrometrie der Androgene bei Frauen) erklärt werden. Der Zusammenhang zwischen SHBG und BMI wurde in mehreren Studien nachgewiesen (17) und es stellt sich die Frage, ob der BMI hinsichtlich des Zusammenhangs zwischen SHBG und MetS wie auch T2DM eine Mediatorrolle einnimmt. Unter anderem wurde in einer Studie, die die Mendelian-Randomization-Methode nutzte, gezeigt, dass SHBG sowohl im Insulin- als auch im Glukosemetabolismus eine direkte Rolle zukommt (14, 73). Gleichzeitig scheint prädiabetische Hyperinsulinämie die SHBG-Produktion zu senken. In der vorliegenden und anderen früheren Studien (68) ist die Assoziation zwischen SHBG und MetS jedoch unabhängig vom BMI/Taillenumfang und auch anderen möglichen Einflussfaktoren, wie Verhaltensrisikofaktoren und Komorbiditäten, was auf einen

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unabhängigen Zusammenhang zwischen SHBG und dem MetS hinweist. Hingegen konnte die im altersadjustierten Modell nachgewiesene Assoziation zwischen SHBG und T2DM nach Adjustierung für den Taillenumfang nicht bestätigt werden. Diese Beobachtungen wurden auch in früheren Studien in dieser Weise nachgewiesen (30).

In dieser Studie wurde kein signifikanter Zusammenhang zwischen Sexualhormonen/SHBG und inzidenten, klinisch manifestierten KVK beobachtet. Das entspricht Ergebnissen aus anderen Längsschnittstudien mit weiblicher Studienpopulation (69). Während bei Männern niedrige T-Konzentrationen mit der KVK- oder Gesamt-Mortalität im Zusammenhang stehen (74, 75), konnten in der vorliegenden Arbeit und weiteren Studien (76, 77) mit weiblichen Studienpopulationen keine Assoziation zwischen Sexualhormonen/SHBG und Mortalität beobachtet werden. Andererseits wurde bei 875 postmenopausalen diabetischen Frauen ein Zusammenhang zwischen fT und der KVK- sowie der Gesamt-Mortalität postuliert (47). Außerdem waren extrem hohe T-Konzentrationen in einer Studie mit 4716 dänischen Frauen mit der Mortalität assoziiert (71). Die widersprüchliche Studienlage lässt sich größtenteils mit Interstudien-Variabilität erklären. So wurden die meisten Studien mit älteren oder ausschließlich postmenopausalen Frauen durchgeführt (47), die Sexualhormone/SHBG wurden mit verschiedenen Methoden gemessen und kardiovaskuläre Outcomes unterschiedlich definiert (71). Außerdem wurden Ausschlüsse von Probanden nicht einheitlich vorgenommen und die Analysen für verschiedene Kofaktor adjustiert. Die meisten früheren Studien beobachteten zusammenfassend keinen direkten Zusammenhang zwischen Sexualhormonen/SHBG und klinisch manifestierten KVK oder der Mortalität, hingegen gab es Hinweise darauf, dass direkte oder indirekte Zusammenhänge zwischen Sexualhormonen und dem KVK-Risiko bestehen.

So haben Studien gezeigt, dass hohe Androgenkonzentrationen mit dem KVK-Risiko bei Frauen assoziiert sind (67, 78, 79), obwohl sie aber keinen direkten Einfluss auf das KVK-Risiko zu haben scheinen, sondern als Mediator für beispielsweise die sich verschlechternde endotheliale Funktion fungieren (80). In der vorliegenden Studie wurden beim Vergleich von prä- und postmenopausalen Frauen, abgesehen von der Dyslipidämie, keine Unterschiede in den Assoziationen zwischen Androgenkonzentrationen und kardiometabolischen Outcomes beobachtet. Diese Ergebnisse stützen die Hypothese, dass Androgenkonzentrationen mit dem Alter fallen, aber keinen signifikanten Bezug zur Menopause haben (69). Hauptsächlich scheint das chronologische Altern und nicht das Altern der Ovarien die Unterschiede in den zirkulierenden Androgenkonzentrationen zu beeinflussen (69). Andererseits ist bekannt, dass

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das postmenopausale Ovar weiterhin Androgene sekretiert, die zusammen mit den adrenalen Androgenen einen pleiotropen Effekt auf die Entwicklung von KVK haben. Neben der Rolle der hohen Androgenkonzentrationen für ein ungünstiges kardiovaskuläres Risikoprofil bei Frauen werden interessanterweise auch niedrige Androgenkonzentrationen diskutiert. So würde sich eine U-förmige Beziehung zwischen Androgenen und kardiovaskulärem Outcomes ergeben, wie sie schon bei Männern angenommen wurde (81). Bei postmenopausalen Frauen bestätigen einige Studien den in dieser Studie gezeigten positiven Zusammenhang zwischen endogenen Androgenen und kardiovaskulären Risikofaktoren (67, 78, 79). Gleichzeitig wiesen andere Studien einen linearen Zusammenhang zwischen FAI und dem KVK-Risiko nach, was bedeuten würde, dass Frauen mit niedrigen Androgenen ein günstigeres kardiovaskuläres Profil zeigen. Im Gegensatz dazu haben aber Frauen mit prämaturer Ovarialinsuffizienz, d.h. mit niedrigen Androgenkonzentrationen, ein erhöhtes KVK-Risiko verglichen mit gesunden Frauen (69). Somit spricht diese Studienlage zusammenfassend für eine U-förmige Beziehung zwischen Androgenen und dem kardiovaskulären Risiko bei Frauen. Diese Art des Zusammenhangs könnte in weiterführenden Analysen der SHIP zukünftig untersucht werden.

4.2 Stärken und Limitationen der Studie

Die Stärken der Studie umfassen die ausführlichen Phänotypisierungen der großen populationsbasierten Studienpopulation, welche ausschließlich durch geschultes Personal und basierend auf standardisierten Untersuchungsprotokollen durchgeführt wurde. Zusätzlich stellt die Messung der Androgene anhand der Massenspektrometrie eine weitere Stärke dar. Mit ihr können die sehr niedrigen Androgenkonzentrationen der Frau im Vergleich zu dem sonst häufig genutzten Immunoassay mit deutlich geringerer Intra- und Interlaborvariabilität und einer höheren Sensitivität gemessen werden (48, 49).

Eine wesentliche Limitation der Längsschnittanalysen ist, dass die Sexualhormone/SHBG ausschließlich in der Basisuntersuchung gemessen wurden. Unter diesen Bedingungen muss die Annahme getroffen werden, dass die Hormonkonzentrationen in der Studienpopulation über den Follow-up Zeitraum konstant bleiben. Dies ist aber vor allem im Verlauf des Alterungsprozesses (7, 69) und der Übergangsphase von prä- zum postmenopausalen Status nicht der Fall (6) und kann daher zu einer Verzerrung der Analyse geführt haben. Eine Limitation der Analyse zum MetS ergibt sich aus den Blutproben der nicht nüchternen Probanden, da die Definition des MetS auf nüchternen Blutproben beruht. Die in der vorliegenden Arbeit verwendete Definition des MetS wurde deshalb anhand einer Studie über

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Risikofaktoren für KVK und deren Schwellenwerte von 6805 Blutproben nicht nüchterner, schwedischer Frauen korrigiert (59). Die Ideallösung einer vollständig nüchternen Studienstichprobe war auf Grund der Größe der Studienpopulation organisatorisch und logistisch nicht umzusetzen. Eine weitere Limitation sind die unzureichenden Angaben zum Ovarialzyklus der weiblichen Probanden. Da die Gonadenhormone während des weiblichen Zyklus erheblich schwanken, wäre eine Adjustierung für den Zeitpunkt im Ovarialzyklus bei allen Frauen ein geeignetes Mittel, um Verzerrungen der Analysen vor allem für Estrogene zu verringern. Allerdings sind auch in der früheren Literatur große Interstudien-Differenzen bezüglich des Umganges mit dem Ovarialzyklus zu finden (82), so dass es für den Vergleich von Studien von großem Nutzen wäre, eine allgemeine Definition zur Feststellung des Zeitpunktes im Ovarialzyklus zu etablieren.

4.3 Ausblick

Die vorliegende Arbeit konnte bei gesunden Frauen aus der Allgemeinbevölkerung vielfache Zusammenhänge zwischen Sexualhormonen/SHBG mit kardiometabolischen Risikofaktoren belegen. Ergebnisse von Beobachtungsstudien im Allgemeinen und epidemiologischer Forschung im Speziellen lassen keine Rückschlüsse auf eine mögliche Kausalität der beobachteten Zusammenhänge zu (83). Darüber hinaus waren fast alle beobachteten Zusammenhänge lediglich in den Querschnittsanalysen signifikant, die in keinerlei Hinsicht Schlussfolgerungen zu Ursache und Wirkung zulassen. Epidemiologische Studien spielen daher vielmehr eine Rolle bei der Entdeckung neuer Biomarker. Aus den Ergebnissen der vorliegenden Arbeit kann demnach nicht gefolgert werden, ob Sexualhormone/SHBG lediglich den aktuellen metabolischen Zustand spiegeln oder sich auch direkte kausale Zusammenhänge dahinter verbergen. Frühere Studien mit männlicher Studienpopulation (9) lassen aber die Hypothese zu, dass die veränderten Sexualhormonkonzentrationen als Biomarker bzw. *Risikomarker* für KVK oder deren Risikofaktoren betrachtet werden können, die im Gegensatz zu *Risikofaktoren* keine ursächliche Rolle für die Entstehung der jeweiligen Erkrankung spielen (84). Auf Grund der hier vorliegenden Ergebnisse scheint vor allem SHBG als Biomarker für das MetS, besonders bei postmenopausalen Frauen, ein gewisses Potential zur Risikoprädiktion zu besitzen. Weiterführend könnten zukünftige Studien ein besonderes Augenmerk auf die Analyse potentieller Biomarker vor allem für die Risikozeit während der Menopause legen, da postmenopausale Frauen ein 2,6-faches Risiko für KVK verglichen mit prämenopausalen Frauen gleichen Alters aufweisen (85).

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Die Beantwortung der Frage nach der klinischen Bedeutsamkeit der hier beobachteten Zusammenhänge erfordert weitere Ergebnisse populationsbasierter Kohortenstudien und zusätzlichen, klinischen, randomisiert-kontrollierten Studien mit weiblicher Studienpopulation, besonders da Frauen im Vergleich zu Männern in diesen Studien derzeit noch unterrepräsentiert sind (86). Insbesondere der Vergleich zu anderen großen Kohortenstudien wäre interessant, da die vorliegenden Arbeit auf der SHIP basiert, deren nordostdeutsche Studienpopulation durch eine erhöhte Prävalenz für Verhaltensrisikofaktoren (Alkohol- und Tabakkonsum), subklinische (Hypertonie, Fettleibigkeit) und klinische KVK gekennzeichnet ist und eine geringere Lebenserwartung im Vergleich zu anderen deutschen Regionen aufweist (87).

Die in früheren langjährigen Beobachtungsstudien und teilweise in der vorliegenden Studie beobachteten möglichen protektiven kardiovaskulären Effekte endogener Sexualhormone/SHBG konnten allerdings in klinischen Interventionsstudien bislang nicht überzeugend belegt werden. In der Women's Health Initiative, der HERS (88) sowie der ESPRIT-Studie (89) konnte kein protektiver Effekt einer HT auf inzidente koronare Herzerkrankungen nachgewiesen werden (90) und es wurde in Frage gestellt, ob die HT zur Sekundärprävention kardiovaskulärer Ereignisse geeignet ist. Zusammenfassend kann beobachtet werden, dass das KVK-Risiko nach der Menopause deutlich ansteigt und Zusammenhänge zwischen Sexualhormonen und dem kardiovaskulären Risiko in epidemiologischen Studien nachgewiesen werden konnten. Gleichzeitig gibt es momentan aber keine aussagekräftigen hochqualitativen Daten aus klinischen Studien, die belegen, dass exogene Sexualhormone einen positiven Einfluss auf das KVK-Risiko haben. Zukünftige Studien könnten den Unterschied der Effekte von endogenen und exogenen Sexualhormonen in den Fokus stellen. Außerdem sollte der Sexualhormon/SHBG-Metabolismus in seiner Gesamtheit als komplexes System betrachtet werden, da die Diskrepanzen der bisherigen Studien auch auf eine fehlende Berücksichtigung des Zusammenwirkens der Sexualhormone untereinander zurückzuführen sein könnten (70). T und E2 sind im Serum größtenteils an SHBG gebunden, wobei SHBG jedoch T mit einer höheren Affinität bindet. Das heißt, dass erhöhte SHBG-Konzentrationen bei gleichbleibenden T- und E2-Konzentrationen einen relativen Anstieg des freien E2 verglichen mit fT ergeben (69). Vor diesem Hintergrund wäre es von Interesse, einen Standard für die Adjustierung bei Analysen von Sexualhormonen zu etablieren, wobei beispielsweise Analysen mit Sexualhormonen stets für SHBG adjustiert werden könnten. In einigen früheren Studien wurde dies bereits praktiziert, allerdings vor allem in Hinblick auf das Zusammenspiel zwischen SHBG und der Outcome-Variable (91).

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Als Erweiterung der vorliegenden Arbeit wäre es in nachfolgenden Analysen in der SHIP außerdem aufschlussreich, den Zusammenhang zwischen Estrogenen und kardiovaskulären Risikofaktoren auch im Längsschnitt zu analysieren. Auf Grund fehlender Daten war dies in der vorliegenden Arbeit noch nicht möglich, kann aber nach Abschluss der Untersuchungen 2017 in SHIP-TREND-1 angestrebt werden. Hinsichtlich der Vergleichbarkeit von Studien, die Sexualhormone bei Frauen analysieren, wäre es sinnvoll standardisierte Messmethoden für Androgene bei Frauen zu etablieren (92).

Grundsätzlich ist die Forschung zu KVK gerade zu diesem Zeitpunkt relevant, da sich die Lebensdauer in den Industriestaaten immer weiter verlängert und gleichzeitig die Geburtenrate sinkt. KVK als altersassoziierte Erkrankung stellt bereits heute die häufigste Todesursache dar (42) und ihr wird mit der alternden Gesellschaft eine immer größere Rolle zukommen. Weitere Biomarker für KVK-Morbidität und -Mortalität zu identifizieren, wäre bei der Prävention dieser Krankheiten eine Hilfe - auch, weil Biomarker relativ leicht verfügbar, kosteneffizient und in weiten Teilen der Medizin akzeptiert sind (93).

5. Zusammenfassung

5. Zusammenfassung

In der vorliegenden Arbeit wurden Zusammenhänge zwischen Sexualhormonen/SHBG und einem breiten Spektrum kardiovaskulärer Risikofaktoren, Krankheiten und Mortalität in einer gesunden weiblichen Allgemeinbevölkerung in Nordostdeutschland untersucht.

Krankheiten des Herz-Kreislaufsystems sind die häufigste Todesursache bei Frauen weltweit. Risikofaktoren für kardiovaskuläre Krankheiten schließen den Typ 2 Diabetes mellitus, Übergewicht, Hypertonie und Fettwechselstörungen ein. Das gemeinsame Auftreten von definierten, multiplen und metabolischen Veränderungen wird als das Metabolische Syndrom bezeichnet. Zusätzlich weisen subklinische Veränderungen des kardiovaskulären Systems auf ein erhöhtes Risiko für klinisch manifestierte, kardiovaskuläre Krankheiten hin.

Es wurden Daten der populationsbasierten longitudinalen *Study of Health in Pomerania* herangezogen und rund 2000 Frauen im Alter zwischen 20 und 79 Jahren analysiert. Um die Assoziation zwischen Sexualhormonen und kardiovaskulären Risikofaktoren sowie Mortalität zu untersuchen, wurden verschiedene multivariable Regressionsmodelle verwendet.

Die Ergebnisse zeigen, dass die untersuchten Sexualhormone/SHBG mit verschiedenen klinischen Korrelaten wie zum Beispiel BMI, Blutdruck oder Lipoproteinen in Beziehung stehen. Außerdem konnte nachgewiesen werden, dass SHBG, unabhängig von relevanten Kofaktoren, mit prävalentem und inzidentem Metabolischem Syndrom sowie prävalentem Typ 2 Diabetes mellitus assoziiert ist. Es wurde kein unabhängiger Zusammenhang zwischen Sexualhormonen/SHBG mit inzidenten subklinischen oder klinischen kardiovaskulären Krankheiten oder der Mortalität gefunden. Die meisten dargestellten Ergebnisse bestätigen frühere internationale Studien und erweitern sie um den Aspekt der großen weiblichen Studienstichprobe. Für die zukünftige Forschung wäre es von großem Interesse, das prädiktive Potential von SHBG als Biomarker des Metabolischen Syndroms in anderen populationsbasierten bzw. patientenbasierten Studien zu bestätigen, um somit neue Biomarker für kardiovaskuläre Krankheiten zu etablieren.

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7. Anhang

A Wissenschaftliche Leistungen

Originalartikel

Benjamin Fenske*, Hanna Kische*, Stefan Gross, Henri Wallaschofski, Henry Völzke, Marcus Dörr, Matthias Nauck, Brian G. Keevil, Georg Brabant, Robin Haring. Endogenous Androgens and Sex Hormone–Binding Globulin in Women and Risk of Metabolic Syndrome and Type 2 Diabetes. *J Clin Endocrinol Metab.* 2015;100(12):4595-603.

* both authors contributed equally.

Gotja Schaffrath*, Hanna Kische*, Stefan Gross, Henri Wallaschofski, Henry Völzke, Marcus Dörr, Matthias Nauck, Brian G. Keevil, Georg Brabant, Robin Haring. Association of sex hormones with incident 10-year cardiovascular disease and mortality in women. *Maturitas.* 2015;82(4):424-30.

* both authors contributed equally.

Hanna Kische, Stefan Gross, Henri Wallaschofski, Henry Völzke, Marcus Dörr, Matthias Nauck, Stephan B. Felix, Robin Haring. Serum androgen concentrations and subclinical measures of cardiovascular disease in men and women. *Atherosclerosis.* 2016;247:193-200.

Hanna Kische, Stefan Gross, Henri Wallaschofski, Henry Völzke, Marcus Dörr, Matthias Nauck, Robin Haring. Clinical correlates of sex hormones in women: the Study of Health in Pomerania. *Metabolism.* 2016;65(9):1286-96.

Hanna Kische, Ralf Ewert, Ingo Fietze, Stefan Gross, Henri Wallaschofski, Henry Völzke, Marcus Dörr, Matthias Nauck, Anne Obst, Beate Stubbe, Thomas Penzel, Robin Haring. Sex Hormones and Sleep in Men and Women from the General Population: A Cross-Sectional Observational Study. *J Clin Endocrinol Metab.* 2016;101(11): 3968-3977.

Hanna Kische, Robin Haring. Androgenkonzentrationen und kardiovaskuläre Risiken bei der Frau. *Der Gynäkologe.* 2016. DOI 10.1007/s00129-016-3937-7.

Hanna Kische, Andreas Arnold, Stefan Gross, Henri Wallaschofski, Henry Völzke, Matthias Nauck, Prof. Robin Haring. Sex hormones and hair loss in men from the general population. JAMA Dermatology. *in press*

Konrad Pätzug, Nele Friedrich, Hanna Kische, Anke Hannemann, Henry Völzke, Matthias Nauck, Brian G. Keevil, Robin Haring. Sex hormones and bone stiffness in men and women from the general population. Bone. *under review*

Tom Seyfart, Nele Friedrich, Hanna Kische, Henri Wallaschofski, Henry Völzke, Matthias Nauck, Brian G. Keevil, Robin Haring. Association of sex hormones with anthropometry assessed by physical, laboratory, and imaging markers in men and women from the general population. Maturitas. *under review*

Poster

Hanna Kische, Ralf Ewert, Ingo Fietze, Stefan Gross, Henri Wallaschofski, Henry Völzke, Marcus Dörr, Matthias Nauck, Anne Obst, Beate Stubbe, Thomas Penzel, Robin Haring. Sex Hormones and Sleep in Men and Women from the General Population: A Cross-Sectional Observational Study. Boston, 2016: The Endocrine Society, Jahrestagung.

Hanna Kische, Ralf Ewert, Ingo Fietze, Stefan Gross, Henri Wallaschofski, Henry Völzke, Marcus Dörr, Matthias Nauck, Anne Obst, Beate Stubbe, Thomas Penzel, Robin Haring. Sex Hormones and Sleep in Men and Women from the General Population: A Cross-Sectional Observational Study. München, 2016, European Congress of Endocrinology.

B Danksagungen

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Mein besonderer Dank gilt Herrn Prof. Dr. rer. med. Robin Haring für die Vergabe des Promotionsthemas, die freundliche und geduldige Betreuung, sowie die Unterstützung bei den wissenschaftlichen Analysen.

Ich bedanke mich bei Prof. Dr. med. Henri Wallaschofski, Prof. Dr. med. Marcus Dörr und bei Prof. Dr. med. Henry Völzke und bei allen weiteren Koautoren für die konstruktive Kritik und die gute Zusammenarbeit im Rahmen der Entstehung der Publikationen. Dr. Stefan Gross sei für die Hilfe beim Erweitern der Kenntnisse des Stata-Programmes gedankt. Allen Kollegen in meiner Arbeitsgruppe möchte ich für ein entspanntes und konstruktives Arbeitsklima danken.

Von ganzem Herzen danke ich meiner Familie für ihre Unterstützung und ihr Verständnis. Schließlich danke ich allen Probanden der SHIP-Studie, deren Teilnahme den Grundstein der vorliegenden wissenschaftlichen Arbeiten gelegt hat.

C Begleitende wissenschaftliche Arbeiten

Die Publikationen der vorliegenden Promotionsschrift, werden in der Reihenfolge aufgeführt, in der sie in der vorliegenden Arbeit vorgestellt wurden.

Hanna Kische, Stefan Gross, Henri Wallaschofski, Henry Völzke, Marcus Dörr, Matthias Nauck, Robin Haring. *Clinical correlates of sex hormones in women: the Study of Health in Pomerania*. Metabolism. 2016; 65(9): 1286-96.

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* both authors contributed equally.

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Clinical correlates of sex hormones in women: The study of health in Pomerania



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ARTICLE INFO

Article history:

Received 15 February 2016

Accepted 17 May 2016

Keywords:

Testosterone

Estrogens

Sex hormones

Women

Clinical correlates

ABSTRACT

Background. Despite associations of sex hormones in women with increased cardiometabolic risk and mortality, the clinical correlates of altered sex hormone concentrations in women are less clearly understood. We investigated a broad range of clinical correlates of sex hormones in women from a large population-based sample.

Methods. Data from 2560 women from two cohorts of the Study of Health in Pomerania were used. Stepwise multivariable regression models were implemented to investigate a broad range of behavioral, socio-demographic, and cardiometabolic clinical correlates related to total testosterone (TT), free testosterone (fT), androstenedione (ASD), dehydroepiandrosterone-sulfate (DHEAS), estrone (E1), estradiol (E2), and sex hormone-binding globulin (SHBG).

Results. Waist circumference and BMI (β -coefficient: -0.03 ; 95% CI: -0.04 ; 0.03) were inversely related to SHBG, and BMI was positively related to TT (β -coefficient: 0.005 ; 95% CI: 0.001 ; 0.009), fT, E1, and E2. Smoking was positively related to TT (β -coefficient: 0.04 ; 95% CI: 0.01 ; 0.06), ASD, and fT. Systolic blood pressure (TT: β -coefficient: 0.002 ; 95% CI: 0.001 ; 0.003), hypertension (TT: β -coefficient: 0.05 ; 95% CI: 0.003 ; 0.11), low-density lipoprotein (LDL) cholesterol (TT: β -coefficient: 0.02 ; 95% CI: 0.01 ; 0.05), and total cholesterol (TT: β -coefficient: -0.03 ; 95% CI: 0.01 ; 0.05) were positively related to TT and ASD. Finally, type 2 diabetes mellitus (T2DM), and metabolic syndrome (MetS) were positively related to fT, but inversely related to SHBG.

Conclusions. Our population-based study, with sex hormone concentrations measured by liquid chromatography tandem mass spectrometry, revealed associations between clinical correlates including waist circumference, smoking, cohabitation, systolic blood pressure, cholesterol, and MetS with sex hormones. Thus, sex hormones and SHBG may play a role in the cardiovascular risk profile of women.

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Abbreviations: ASD, androstenedione; BMI, body mass index; CVD, cardiovascular disease; CI, confidence interval; DHEAS, dehydroepiandrosterone-sulfate; E2, estradiol; E1, estrone; fT, free testosterone; LC-MS/MS, liquid chromatography-tandem mass spectrometry; MetS, metabolic syndrome; MV, multivariable; HDL, high-density lipoprotein; PCOS, polycystic ovary syndrome; SHBG, sex hormone-binding globulin; SHIP, Study of Health in Pomerania; TT, total testosterone; T2DM, type 2 diabetes mellitus.

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1. Introduction

The worldwide prevalence of obesity has more than doubled since 1980 [1]. Also in Western Europe, there has been a marked increase in obesity over the past ten years, with a rise in obesity prevalence of 9%–15% [2]. Previous studies have shown that individuals with higher body mass index (BMI) have increased risk of hypertension, diabetes, cardiovascular disease (CVD), and mortality [3]. The role of sex hormones in this context is not entirely clear. Although sex hormone in women have been associated with increased cardiometabolic risk and mortality [4], including a greater prevalence of metabolic syndrome (MetS) [5], type 2 diabetes mellitus (T2DM) [6], hypertension [7], and atherosclerosis [8], the clinical correlates of these hormones are less clearly understood [9]. There is a gap in our current knowledge about whether abnormal sex hormone concentrations are the cause or the consequence of cardiometabolic diseases. Thus, it is important to determine clinical correlates of sex hormones in women.

It has been widely observed that sex hormones in men and women decline with age [10,11] and other contributing factors, e.g. health- and lifestyle-related characteristics [4]. Cross-sectional findings from observational research showed that total testosterone (TT) and dehydroepiandrosterone-sulfate (DHEAS) in women are linked to BMI, MetS, T2DM, and blood pressure [6,9,12], while prospective studies relating sex hormones to incident CVD have yielded inconsistent results [13]. In general, representative studies with women from the general population are rare: Previous studies showed an inverse association between BMI and waist-to-hip-ratio with sex hormone-binding globulin (SHBG). BMI is also positively correlated with total and free estradiol (E2) [10]. Additionally, self-reported health and depressive symptoms were associated with DHEAS [5]. However, these associations were mainly explored in post-menopausal women [4,10]. Other limitations of these previous studies include strongly selected community- or patient-based study samples, small sample sizes, sex hormone measurements by immunoassay, and the selective choice of clinical correlates.

Thus, the association between clinical correlates and sex hormones in women from the general population is largely unknown. To overcome these limitations, we investigated a large, population-based study sample, sex hormone concentrations measured by mass-spectrometry, and a broad range of behavioral, socio-demographic, and cardiometabolic clinical correlates.

2. Methods

2.1. Study Population

The Study of Health in Pomerania (SHIP) is a population-based cohort study in north-eastern Germany. We previously published details of the study design, recruitment, and procedures [14]. The target population of SHIP-0 comprised 6265 eligible individuals (3160 women) with German citizenship and main residency in the study area, including the cities

Greifswald, Stralsund, Anklam, and 29 surrounding communities. At baseline, 4308 individuals (2192 women) aged 20 to 80 years participated between 1997 and 2001, resulting in a response rate of 68.8%. The SHIP-TREND cohort comprised a representative sample of 8826 adults, randomly selected from population registries into 24 age- and sex-specific strata between 2008 and 2012 [14]. In total, 4420 (response 50.1%) individuals participated at baseline. The subsample with available sex hormone data was limited to the first 1000 SHIP-TREND participants that fasted for at least 10 h prior to blood donation. In both cohorts, written informed consent was obtained from each participant and the Ethics Committee of the University of Greifswald authorized the study protocol, which is consistent with the principles of the Declaration of Helsinki. After pooling data from both cohorts ($N = 2708$ women), we excluded women who: received prescribed drugs in the last seven days, based on the medication packages or on self-statement, categorized based on the Anatomical Therapeutic Chemical (ATC) classification index (sex hormone antagonists ($N = 2$), natural opium alkaloids ($N = 39$), and antiandrogens ($N = 15$)); pregnancy ($N = 18$), and self-reported bilateral oophorectomy ($N = 78$). We did not use information about polycystic ovary syndrome (PCOS) as additional exclusion criterion as such self-reported data were not collected. Finally, we investigated a study population of 2560 women (Fig. 1).

2.2. Measures

A computer-assisted personal interview was conducted to assess socio-demographic and behavioral characteristics and medical history including information about sex, age, alcohol consumption, physical training, pregnancy, gynecological surgery, bilateral oophorectomy, and medication use. Mean daily alcohol consumption was calculated using beverage-specific pure ethanol volume proportions. Women with less than one hour physical training per week during winter or summer were defined as physically inactive. As to smoking, women were divided into three categories: current, former, and never-smokers. The sample was stratified into pre- and post-menopausal women applying a previously published categorization: all women <40 years of age and between 40 and 60 years who reported menstrual cycle were classified as pre-menopausal and all women ≥ 60 years of age, together with all women between 40 and 60 years who reported no menstrual cycle were classified as post-menopausal [15]. We assessed the use of oral contraceptive (G03A) and hormone therapy (G03C, G03D, or G03F) based on ATC codes [16]. Standard digital scales were used to measure weight (to the nearest 0.1 kg) of women in light clothing and without shoes. Waist circumference was measured utilizing a tape midway between the lower rib margin and the iliac crest in the horizontal plane (to the nearest 0.1 cm). Height was measured with a digital ultrasound instrument (to the nearest 0.1 cm). BMI was calculated from the body weight in kilogram and height in meters [$BMI = \text{kg}/\text{m}^2$]. After a resting period of at least five minutes systolic and diastolic blood pressures were measured three times on the right arm of seated subjects using an oscillometric digital blood pressure monitor (HEM-705CP, Omron, Tokyo, Japan). The interval between the readings was three minutes and for the present analyses the

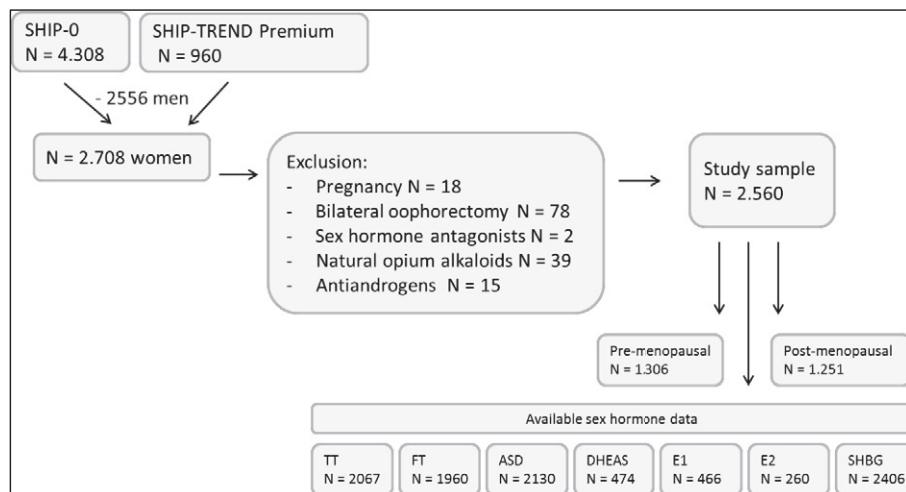


Fig. 1 – Flow chart of the study sample. SHIP Study of Health in Pomerania, TT total testosterone, fT free testosterone, ASD androstenedione, DHEAS dehydroepiandrosterone-sulfate, E1 estrone, E2 estradiol, SHBG sex hormone-binding globulin.

mean of the second and third measurements was used. Hypertension was defined as blood pressure $\geq 140/90$ mmHg or use of antihypertensive medication (ATC codes C02, C03, C04, C07, C08, C09) [17].

Socio-demographic correlates included the following, previously described [9], variables: educational level (<10, 10, or >10 years of schooling), equalized household income (<1000 €, 1000–1500 €, >1500 €), cohabitation (married or living alone), and subjective health (excellent, very good, and good or fair and poor).

CVD was defined based on a previously published [18] summative score comprising diagnosis of angina pectoris, peripheral artery disease, heart failure, stroke, and/or myocardial infarction. T2DM was determined based on self-reported physician's diagnosis, use of antidiabetic medication (ATC code A10) and/or HbA1c concentrations $\geq 6.5\%$ and $<20\%$. MetS components were defined based on waist-circumference ≥ 120 cm, blood pressure $\geq 130/85$ mmHg or self-reported antihypertensive drug treatment, non-fasting glucose ≥ 6.1 mmol/l or antidiabetic treatment (ATC codes A10 A, A10B), non-fasting triglycerides ≥ 1.7 mmol/l or lipid-lowering treatment (ATC codes C10AB, A10 AD), and/or high-density lipoprotein (HDL) cholesterol ≤ 1.3 mmol/l. These diagnostic criteria for the assessment of MetS are premised on the Joint Scientific Statement to harmonize MetS [19] and in SHIP-0 altered for the use of non-fasting blood samples [20]. Dyslipidemia and cancer were identified from self-reported physician's diagnoses. Total cholesterol and HDL cholesterol were measured photometrically (Hitachi 704, Roche Diagnostics, Mannheim, Germany) in SHIP-0 and on the Dimension Vista 500 analytical system (Siemens Healthcare Diagnostics, Eschborn, Germany) in SHIP-TREND. All assays were performed according to the manufacturers' recommendations by skilled technical personnel.

2.2.1. Hormone Measurements

We previously published a detailed description of the performed sex hormone measurements [16]. Briefly, serum aliquots (storage at -80°C) consist of a blood sample, which was taken between 8:00 a.m. and 7:00 p.m. from the cubital

vein in the supine position. Measurements of serum TT, androstenedione (ASD), estrone (E1), and E2 concentrations were carried out in the Department of Clinical Chemistry at the University Hospital of South Manchester (Manchester, UK). Liquid chromatography-tandem mass spectrometry (LC-MS/MS) was performed with a validated routine method [21]. The standard curve was linear up to 50.0 nmol/L, the lower limit of quantitation was 0.25 nmol/L, and intra- and inter-assay coefficients of variation were $<10\%$ for TT and ASD over the range 0.3–35 nmol/L. For E1, the inter-assay imprecision was 5.3, 3.8 and 5.1% and for E2 5.4, 3.7 and 4.9% at concentrations of 125, 400 and 1500 pmol/l, respectively. The intra-assay imprecision for these concentrations was 4.0, 3.4 and 5.0% for E1 and 3.1, 3.5 and 4.0% for E2, respectively. All means were within 8% of the PBS-based quality control targets. The measurement range for E1 and E2 was 25–2000 pmol/l. The lower limit of detection was 3.9 and 8.0 pmol/l for E1 and E2, respectively. The lower limits of quantitation were 6 pmol/l for E1 and 10 pmol/l for E2 [22]. In SHIP-0, SHBG concentrations were measured also from frozen serum aliquots using an Advia Centaur (Siemens Healthcare Diagnostics, Eschborn, Germany) with an inter-assay coefficient of variation of 6.6% at the 27.1 nmol/L level, 7.6% at the 48.2 nmol/L level, and 7.7% at the 52.3 nmol/L level. DHEAS was measured using a competitive chemiluminescent enzyme immunoassay on an Immulite 2500 analyzer (Siemens Healthcare Diagnostics, Eschborn, Germany). In SHIP-TREND, concentrations of SHBG and DHEAS were measured using competitive chemiluminescent immunoassays on an Immulite 2000 XPi (Siemens Healthcare Diagnostics, Eschborn, Germany) with an inter-assay coefficient of variation of 3.5% and 8.3% in the low pool, and 4.8% and 5.4% in the high pool, respectively. Free testosterone (fT) was calculated from measured TT and SHBG concentrations: $[\text{free T (nmol/L)} = ((-\text{a} + \sqrt{\text{b}})/\text{c})/10^{-9}$ with $\text{a} = \text{SHBG (nmol/L)} - \text{TT (nmol/L)} + 23.43$, $\text{b} = \text{a}^2 + (4 \times 23.43 \times \text{TT (nmol/L)})$ and $\text{c} = 2^2 \times 23.43 \times 10^9$ for a standard average albumin concentration of 4.3 g/dL [23].

2.3. Statistical Analysis

Categorical data were given as percentage and continuous data as mean (standard deviation) or median (p25th, p75th).

For intergroup comparisons by menopausal status we used χ^2 test for nominal data and Mann–Whitney-U test for quantitative data. All regression models were performed after log-transforming sex hormone concentrations. We implemented age- and multivariable-adjusted linear regression models with robust standard errors to examine associations of clinical correlates with TT, fT, ASD, DHEAS, E1, E2 and SHBG using sex hormones as the dependent variables. This was followed by a test of normality of regression residuals using QQ plots. Effects were reported as β -coefficients and their 95% confidence interval (CI). Clinical correlates were chosen based on previously reported associations with sex hormones and SHBG [5,9,24–26]. To avoid autocorrelation in the multivariable regression analyses, we aimed to model an adequate minimal adjustment set. Thus, we categorized

potential confounding variables thematically, using the following four different multivariable regression models:

1) "BMI":	age	BMI			
2) "behavior":	age	BMI	smoking	physical inactivity	alcohol consumption
3) "socio-demographic":	age	BMI	net income	cohabitation	educational level
4) "cardio-metabolic":	age	BMI	total cholesterol	triglycerides	HbA1c

All models were additionally adjusted for cohort-affiliation (SHIP-0 or SHIP-TREND). Finally, confounders investigated as independent variable (clinical correlate) were omitted from

Table 1 – Baseline characteristics of the study population.

Variable	All women (N = 2560)	Pre-menopausal Women (N = 1306)	Post-menopausal Women (N = 1251)
Age, years	49.3 (15.6)	37.1 (9.3)	61.8 (9.7)
Total testosterone, nmol/L	0.77 (0.56, 1.05)	0.83 (0.62, 1.14)	0.7 (0.52, 0.96)
SHBG, nmol/L	76.7 (52.9, 113.6)	92.9 (62.6, 141.8)	65.5 (46.7, 90.3)
Free testosterone, nmol/L	0.007 (0.004, 0.011)	0.007 (0.004, 0.011)	0.008 (0.005, 0.012)
Androstenedione, nmol/L	1.77 (1.15, 2.7)	2.42 (1.65, 3.42)	1.33 (0.93, 1.92)
Estrone, nmol/L	120.6 (72.1, 227.8)	189.3 (110.6, 285.4)	86.9 (64.1, 151.3)
Estradiol, nmol/L	222.3 (71.9, 432.7)	261.2 (114.7, 487.7)	157.8 (39.1, 330.2)
DHEAS, mg/L	1.06 (0.68, 1.53)	1.3 (0.9, 1.7)	0.84 (0.55, 1.32)
Body mass index, kg/m ²	26.9 (5.3)	25.4 (5.1)	28.7 (5.3)
Waist circumference, cm	83.2 (12.8)	78.7 (12.0)	87.8 (12.1)
Current smoking, %	25.9	35.9	15.3
Alcohol consumption, g/day	2.9 (0.67, 7.30)	3.95 (1.3, 9.1)	2.1 (0.0, 5.2)
Physically inactive, %	50.8	45.5	56.4
Equalized household income/month			
<1000 €	15.3	14.4	16.2
1000–1500 €	15.1	12.7	17.6
> 1500 €	69.5	72.8	66.1
Educational level			
≤10 classes	31.4	11.2	52.6
> 10 classes	68.5	88.8	47.3
Cohabitation, (living alone)	28.5	22.4	34.8
Self-reported subjective health			
Excellent or very good	18.3	29.1	10.1
Good	64.1	63.1	64.3
Fair or poor	17.6	7.8	25.6
Systolic blood pressure, mmHg	128.1 (20.9)	119.5 (15.8)	136.9 (21.8)
Diastolic blood pressure, mmHg	79.9 (10.7)	78.1 (9.9)	81.8 (11.1)
Hypertension, %	41.8	22.1	60.9
Antihypertensive medication, %	27.9	10.1	44.9
Cardiovascular disease, %	16.4	7.6	25.5
Metabolic syndrome, %	20.6	10.1	31.4
Type 2 diabetes mellitus, * %	9.2	1.3	18.3
Dyslipidemia, %	13.5	2.9	24.6
HDL cholesterol, mmol/L	1.54 (1.31, 1.83)	1.55 (1.33, 1.83)	1.54 (1.27, 1.83)
LDL cholesterol, mmol/L	3.41 (2.7, 4.18)	2.96 (2.4, 3.67)	3.84 (3.18, 4.55)
Cholesterol, mmol/L	5.61 (4.9, 6.45)	5.17 (4.56, 5.27)	6.1 (5.42, 6.9)
Triglycerides, mmol/L	1.27 (0.89, 1.82)	1.08 (0.77, 1.53)	1.48 (1.07, 2.08)
GFR, ml/min per 1.73 m ²	76.7 (67.8, 87.1)	82.8 (74.1, 90.5)	72.5 (63.7, 84.6)
Oral contraceptive use, %	18.7	36.3	0.5
Hormone replacement therapy, %	17.1	7.03	27.7

Data are percentages, mean (SD), or median (Q1, Q3).

Oral contraceptive use was defined according to Anatomical Therapeutic Chemical (ATC) classification code G03AA/B/C/D. Hormone therapy was defined according to Anatomical Therapeutic Chemical (ATC) classification code G03C/D/F.

SHBG, sex hormone-binding globulin; HDL, high-density lipoprotein; LDL, low-density lipoprotein; GFR, glomerular filtration rate.

* Only in the cohort SHIP-0 not in SHIP-TREND (non-diabetic cohort).

the adjustment set of the respective model. Several separate sensitivity analyses were performed. Multiplicative interaction terms between each hormone and covariate were investigated in multivariable models. Given a number of significant interactions terms (p -value <0.05) between hormones and menopausal status, we performed all analyses separately in pre- ($N = 1306$) and post-menopausal ($N = 1251$) women. Further, multivariable regression models were repeated and stratified by use of hormone therapy ($N = 440$) and oral contraceptives ($N = 437$). $P < 0.05$ was considered statistically significant. This manuscript was written in accordance with the STROBE statement, giving guidelines for the reporting of observational studies [27]. All the statistical analyses were performed with Stata 13.0 (Stata, College Station, TX, USA).

3. Results

Table 1 presents the baseline characteristics of the full study sample, as well as stratified by menopausal status. In general, distributions of sex hormone and SHBG concentrations were age-dependent (Supplementary Fig. 1). Results from age- and multivariable-adjusted models of the association between clinical correlates and sex hormones and SHBG are shown in **Table 2**. In age-adjusted models, BMI was inversely related to SHBG and E2, and positively related to TT (β -coefficient: 0.005; 95% CI: 0.001; 0.009), fT, and E1. In all multivariable models (MV-model), waist-circumference was inversely related to SHBG (MV-model 2, β -coefficient: -0.01; 95% CI: -0.02; -0.01). (See **Table 3**.)

With respect to socio-demographic and behavioral variables, multivariable analyses yielded a positive association between smoking and TT (MV-model 2, β -coefficient: 0.04; 95% CI: 0.01; 0.06), ASD, and fT (**Fig. 2**). Physical inactivity and alcohol consumption were inversely associated with fT. Additionally, we found a positive relation between alcohol consumption and DHEAS (MV-model 2, β -coefficient: 0.007; 95% CI: 0.002; 0.01). We detected a positive relation between income and E2 (MV-model 2, β -coefficient: 0.60; 95% CI: 0.29; 0.56). Educational level was inversely associated with TT (MV-model 2, β -coefficient: -0.07; 95% CI: -0.12; -0.01), ASD, and fT in all multivariable models. Cohabitation was inversely associated with TT (MV-model 2, β -coefficient: -0.08; 95% CI: -0.16; -0.09), but positively associated with E1, E2, and DHEAS.

Regarding cardiometabolic variables, systolic blood pressure and hypertension were positively related to TT (MV-model 2, β -coefficient: 0.002; 95% CI: 0.001; 0.003 and 0.05; 95% CI: 0.003; 0.11, respectively), as well as to ASD (MV-model 2, β -coefficient: 0.001; 95% CI: 0.001; 0.002 and 0.05; 95% CI: 0.005; 0.10, respectively). We detected a positive relation between low-density lipoprotein (LDL) and total cholesterol with TT (MV-model 2, β -coefficient: 0.02; 95% CI: 0.01; 0.05 and 0.03; 95% CI: 0.01; 0.05, respectively). Similar associations were observed for ASD, fT, and DHEAS in all models. Accordingly, LDL cholesterol were inversely related to SHBG (MV-model 2, β -coefficient: -0.04; 95% CI: -0.06; -0.02) and E1 (MV-model 2, β -coefficient: -0.09; 95% CI: -0.17; -0.01) (See **Table 3**). Dyslipidemia showed an inverse relation to E2 (MV-model 2, β -coefficient: -0.001; 95% CI: -0.002; -0.001) in all women and in pre-menopausal women a positive relation to fT and DHEAS, as well

as an inverse relation to TT (MV-model 1, β -coefficient: -0.0003; 95% CI: -0.0004; -0.0002), ASD, SHBG, E1, and E2 (Supplementary Table 1). MetS and T2DM showed a positive relation to fT and an inverse relation to SHBG (**Fig. 3**). Finally, sensitivity analyses with the exclusion of women using oral contraceptives or hormone therapy did not substantially alter the revealed estimates (Supplementary Tables 5, 7).

4. Discussion

The present cross-sectional study identified various clinical correlates of sex hormones in women. At this, lipoproteins, hypertension, and smoking were associated with androgens, and waist circumference, lipoproteins, T2DM, and MetS with SHBG. Overall, these findings offer important insights of the interplay between cardiometabolic risk factors, sex hormones and SHBG in women.

In line with previous studies, we observed that BMI was inversely related to SHBG and E2 and positively related to TT, fT, and E1 [28]. As for the relation between BMI and estrogens, decreased estrogen concentrations are associated with an increase in central fat [29]. Additionally, estrogen is supposed to promote accumulation of gluteo-femoral fat [30].

Regarding behavioral variables, the relation between smoking and androgens has been widely investigated in men, suggesting that androgens and SHBG are higher in smokers compared to non-smokers [25]. According to studies reporting higher levels of adrenal key androgens in female smokers [31], we observed positive associations between smoking and ASD and further androgens like TT and fT in women. Possible mechanisms that may explain the androgen-increasing effect of smoking include a decreased androgen metabolism in the liver [32].

Our study revealed associations of alcohol consumption with DHEAS and fT. This positive association is in line with a study among post-menopausal women reporting that alcohol intake is associated with DHEAS [33]. An independent association between alcohol consumption and E1 is additionally observed in a study among post-menopausal women [34], whereas the observed age-adjusted association in our sample was not retained after multivariable adjustment.

Previous studies suggest an inverse association between physical activity and ASD [34], which is consistent with our results. On the contrary, we did not observe a link between physical activity and E2 reported from previous research [34]. However, given the proposed synergy between adiposity and physical activity, and their mutual association to sex hormones [34], BMI and physical inactivity were associated with TT, fT, and SHBG in our study. In summary, the observed multifaceted associations of behavioral correlates with sex hormones and SHBG confirm the suggested impact of health-related lifestyle factors on hormone metabolism in women. Thus, clinical intervention studies with prolonged lifestyle changes significantly modulate sex hormone concentrations [35].

As to socio-demographical correlates, we observed an inverse association between cohabitation and TT, which means that women living alone have higher TT concentrations than married women. This association was already observed in both sexes [24,36] and might be explained by

Table 2 – Multivariable-adjusted models for clinical correlates and sex hormones and SHBG in women.

	TT N = 2067	ASD N = 2130	SHBG N = 2406	fT N = 1960	E1 N = 466	E2 N = 260	DHEAS N = 474
Multivariable models							
Clinical correlates	0 1 2 3 4	0 1 2 3 4	0 1 2 3 4	0 1 2 3 4	0 1 2 3 4	0 1 2 3 4	0 1 2 3 4
Body mass index	+		–	+	+	–	
Waist circumference	+		-----	+++++			
Smoking	+++++	+++++		+++++			
Physical inactivity	---	--	+	----			
Alcohol consumption				----	+		+++++
Income		–	+			+++++	
Educational level	-----	-----		-----			
Cohabitation	-----				+++++	+++	+++++
Subjective health							
Systolic blood pressure	+++++	+++++	–	++	+		
Diastolic blood pressure	+		–	+	+		
Hypertension	+++++	+++++	–	+			
HDL cholesterol	+++++		+++++	-----			
LDL cholesterol	+++++	+++++	-----	+++++	---		+++++
Total cholesterol	+++++	+++++	--	+++++	--		+++++
Triglycerides	----	-----	----	+	--		
Dyslipidemia			--			-----	
HbA1c	+		-----	+++++			
Glucose			-----	+++	+		
Glomerular filtration rate	+++++			+			
Fibrinogen				----	+		+++++
Type 2 diabetes mellitus			-----	+++++			
Cardiovascular disease	–	–					–
Metabolic syndrome			-----	+++++			
Cancer	–						
1 “BMI”							
age	age		age		age		
BMI	BMI		BMI		BMI		
	smoking		net income		total cholesterol		
	physical inactivity		cohabitation		triglyceride		
	alcohol consumption		educational level		HbA1c		

+ significant positive association, – significant inverse association.

Significance level of two-sided probability values $p < 0.05$.

0 is the age-adjusted model. Multivariable models:

Each model is additionally adjusted for cohort affiliation. Any confounder was omitted in the models if the confounder is equal to the clinical correlate. TT, total testosterone; ASD, androstenedione; SHBG, sex hormone-binding globulin; fT, free testosterone; E1, estrone; E2, estradiol; BMI, body mass index; LDL, low-density lipoprotein; HDL, high-density lipoprotein.

potentially increased quality of marital interaction, decreased marital conflicts, and additionally facilitated parental care [37].

Regarding cardiometabolic variables, we observed a significant positive relation between systolic blood pressure and hypertension with TT and ASD after multivariable adjustment. Whereas previous studies in post-menopausal or hypertensive women reported a stronger effect of systolic blood pressure and hypertension on fT than TT [38], we observed a stronger relation between hypertension and TT. At this, effects of androgens on blood pressure are controversially discussed. In men, some studies found a positive and some an inverse relation between blood pressure and TT [9,39]. There is growing evidence that androgens are related to CVD also in women, but whether androgens have an independent effect on hypertension is not clearly understood to date [40]. However, as shown in previous studies, the relation of systolic blood pressure and hypertension with TT and ASD appears to be independent [41], but the mechanism is not completely understood. In castrated female rats, hypertension can be initiated by treatment with

testosterone [42]. Nevertheless removal of the gonads reduces blood pressure only in hypertensive male rats, but not in females. As a suggested mechanism, androgens might increase blood pressure by directly up-regulating the proximal tubule renin-angiotensin system but down-regulating volume reabsorptive rate [43]. Accordingly, a study in post-menopausal women showed that endothelial dysfunction is associated with increased fT concentrations [44].

A link between lipoproteins and total cholesterol with sex hormones has been suggested in previous studies showing that post-menopausal women have higher total cholesterol and LDL cholesterol concentrations and lower HDL cholesterol concentrations than pre-menopausal women [26]. Moreover, clinical studies found that hormone therapy/treatment with E2 resulted in reduced total cholesterol and LDL cholesterol in post-menopausal women and increased HDL cholesterol concentrations in dyslipidemic or glucose intolerant post-menopausal women [45–47]. Consistent with previous research [46], we observed an inverse relation of LDL cholesterol and total

Table 3 – Multivariable-adjusted models for clinical correlates and sex hormones and SHBG in women.

Beta-coefficient (95% CI)

	Total Testosterone	Androstenedione	SHBG	Free Testosterone	Estrone	Estradiol	DHEAS
Waist circumference	-0.003 (-0.007; 0.001)	0.001 (-0.001; 0.005)	-0.01 (-0.02; -0.01)*	0.01 (0.005; 0.01)*	-0.003 (-0.01; 0.009)	-0.004 (-0.02; 0.01)	0.002 (-0.006; 0.01)
Smoking	0.04 (0.01; 0.06)*	0.08 (0.05; 0.11)*	-0.01 (-0.04; 0.01)	0.05 (0.02; 0.09)*	0.01 (-0.07; 0.09)	0.05 (-0.09; 0.20)	-0.001 (-0.06; 0.06)
Physical inactivity	-0.04 (-0.08; -0.001)*	-0.02 (-0.06; 0.01)	0.01 (-0.02; 0.06)	-0.06 (-0.12; -0.009)*	0.008 (-0.14; 0.15)	0.10 (-0.16; 0.36)	-0.01 (-0.12; 0.09)
Alcohol consumption	-0.001 (-0.004; 0.001)	-0.001 (-0.003; 0.0003)	0.0006 (-0.002; 0.004)	-0.003 (-0.006; -0.001)*	0.004 (-0.002; 0.01)	0.001 (-0.01; 0.01)	0.007 (0.002; 0.01)*
Income	-0.007 (-0.07; 0.06)	0.03 (-0.07; 0.06)	-0.06 (-0.14; -0.006)	0.04 (-0.03; 0.15)	0.28 (-0.07; 0.44)	0.60 (0.29; 0.56)*	0.009 (-0.11; 0.13)
Educational level	-0.07 (-0.12; -0.01)*	-0.07 (-0.12; -0.02)*	0.01 (-0.03; 0.07)	-0.10 (-0.17; -0.02)*	0.02 (-0.18; 0.22)	0.10 (-0.26; 0.46)	-0.03 (-0.21; 0.14)
Cohabitation	-0.08 (-0.16; -0.09)*	0.01 (-0.05; 0.09)	0.02 (-0.05; 0.11)	-0.07 (-0.18; 0.03)	0.34 (0.16; 0.53)*	0.45 (0.04; 0.88)*	0.17 (0.02; 0.33)*
Subjective health	0.02 (-0.04; 0.28)	0.001 (-0.06; 0.06)	-0.006 (-0.06; 0.04)	0.02 (-0.05; 0.10)	0.04 (-1.23; 0.32)	-0.10 (-0.65; 0.43)	-0.15 (-0.35; 0.03)
Systolic blood pressure	0.002 (0.001; 0.003)*	0.001 (0.001; 0.002)*	0.0008 (-0.001; 0.002)	0.002 (0.0003; 0.003)*	0.001 (-0.003; 0.006)	0.006 (-0.004; 0.01)	0.003 (-0.0001; 0.006)
Diastolic blood pressure	0.002 (-0.001; -0.004)	0.001 (-0.001; 0.004)	0.001 (-0.001; 0.003)	0.002 (-0.0008; 0.005)	0.002 (-0.005; 0.009)	0.009 (-0.006; 0.02)	0.004 (-0.0005; 0.009)
Hypertension	0.05 (0.003; 0.11)*	0.05 (0.005; 0.10)*	-0.01 (-0.06; 0.04)	0.06 (0.002; 0.13)*	0.03 (-0.14; 0.21)	0.07 (-0.30; 0.46)	-0.04 (-0.17; 0.08)
HDL-cholesterol	0.10 (0.05; 0.16)*	0.02 (-0.02; 0.06)	0.27 (0.19; 0.34)*	-0.11 (-0.17; -0.04)*	0.06 (-0.14; 0.27)	0.05 (-0.39; 0.50)	-0.01 (-0.15; 0.13)
LDL-cholesterol	0.02 (0.01; 0.05)*	0.03 (0.01; 0.05)*	-0.04 (-0.06; -0.02)*	0.06 (0.03; 0.09)*	-0.09 (-0.17; -0.01)*	-0.05 (-0.21; 0.10)	0.08 (0.02; 0.14)*
Total cholesterol	0.03 (0.01; 0.05)*	0.02 (0.003; 0.04)*	-0.006 (-0.02; 0.01)	0.03 (0.01; 0.06)*	-0.06 (-0.13; -0.002)*	-0.02 (-0.16; -0.11)	0.07 (0.02; 0.12)*
Triglycerides	-0.03 (-0.05; -0.01)*	-0.03 (-0.04; -0.01)*	-0.04 (-0.07; -0.007)*	-0.002 (-0.02; 0.02)	-0.08 (-0.19; 0.01)	0.01 (-0.19; 0.23)	0.03 (-0.04; 0.12)
Dyslipidemia	-0.0001 (-0.001; 0.002)	-0.0001 (-0.0004; 0.0003)	-0.0002 (-0.0004; 0.0001)	0.0001 (-0.001; 0.004)	-0.0002 (-0.001; 0.001)	-0.001 (-0.002; -0.001)*	0.0001 (-0.001; 0.001)
HbA1c	0.008 (-0.02; 0.04)	0.02 (-0.006; 0.05)	-0.09 (-0.12; -0.05)*	0.09 (0.05; 0.13)*	-0.10 (-0.24; 0.03)	-0.21 (-0.48; 0.05)	0.03 (-0.07; 0.14)
Glucose	-0.002 (-0.02; 0.01)	0.008 (-0.01; 0.02)	-0.02 (-0.04; -0.01)*	0.02 (0.002; 0.05)*	0.03 (-0.07; 0.14)	-0.13 (-0.39; 0.13)	0.05 (-0.02; 0.12)
Glomerular filtration rate	0.001 (-0.001; 0.002)	0.002 (0.001; 0.003)*	-0.0002 (-0.001; 0.001)	0.001 (-0.0005; 0.003)	0.001 (-0.002; 0.004)	0.005 (-0-001; 0.01)	-0.001 (-0.003; 0.001)
Fibrinogen	-0.01 (-0.03; 0.003)	-0.009 (-0.02; 0.008)	0.04 (-0.002; 0.08)	-0.04 (-0.07; -0.01)*	0.04 (-0.05; 0.15)	0.17 (-0.04; 0.38)	0.08 (0.002; 0.16)*
Type 2 diabetes mellitus	-0.01 (-0.11; 0.07)	0.03 (-0.04; 0.12)	-0.21 (-0.30; -0.12)*	0.17 (0.05; 0.28)*	-0.25 (-0.56; 0.04)	-0.57 (-0.19; 0.05)	-0.05 (-0.40; 0.29)
Cardiovascular disease	-0.01 (-0.08; 0.05)	-0.04 (-0.11; 0.02)	-0.009 (-0.06; 0.04)	-0.01 (-0.09; 0.06)	-0.04 (-0.29; 0.19)	-0.17 (-0.64; 0.29)	-0.25 (-0.46; -0.05)*
Metabolic syndrome	-0.04 (-0.10; 0.01)	-0.003 (-0.05; 0.05)	-0.22 (-0.28; -0.16)*	0.12 (0.05; 0.20)*	0.04 (-0.14; 0.24)	-0.15 (-0.54; 0.24)	0.09 (-0.05; 0.24)
Cancer	-0.04 (-0.14; 0.05)	-0.02 (-0.09; 0.04)	-0.05 (-0.13; 0.02)	0.01 (-0.09; 0.12)	0.33 (-0.008; 0.68)	0.23 (-0.89; 0.51)	0.06 (-0.21; 0.33)

Data are β-coefficients and their 95% confidence interval with p < 0.05 marked as *.

In this table we used the multivariable model 2 ("behavior") and it was adjusted for age, cohort-affiliation, body mass index, smoking status (three categories), physical inactivity, alcohol consumption. Confounders investigated as independent variable (clinical correlate) were omitted from the adjustment set of the respective model.

Reference categories: educational level: < 10 years vs. > 10 years; subjective health: excellent, good and very good vs. poor and fair; cohabitation: living alone vs. married.

DHEAS, dehydroepiandrosterone-sulfate; SHBG, sex hormone-binding globulin; CI, confidence interval, LDL, low-density lipoprotein; HDL, high-density lipoprotein.

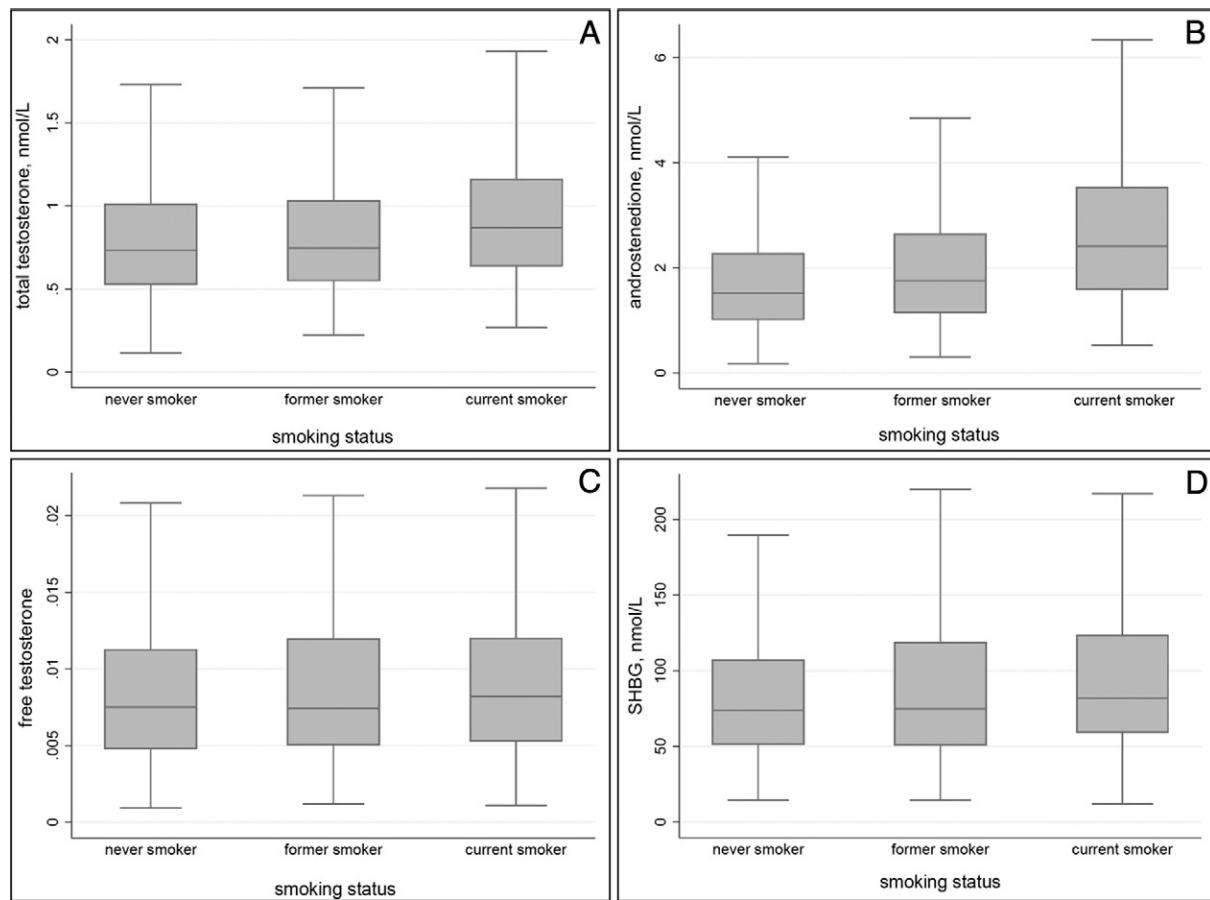


Fig. 2 – Associations between total and free testosterone, androstenedione and SHBG with smoking. Box plots of associations between A) total testosterone, B) androstenedione, C) free testosterone, and D) sex hormone-binding globulin (SHBG) with smoking. A), B), and C) are significant associations. Whiskers are defined as the most extreme values within the upper quartile + 1.5 (Q3–Q1) and the lower quartile – 1.5 (Q3–Q1), respectively.

cholesterol with E1. Additionally, we detected the previously reported positive association between HDL cholesterol and TT [48]. Interestingly, we observed the strongest effects of dyslipidemia on sex hormones among pre-menopausal women, which are consistent with studies among men, identifying young and

middle-aged men with low TT concentrations at the highest risk for incident dyslipidemia [49].

Associations of MetS and T2DM with SHBG and fT were observed in the present study, as well as in previous studies [5,50,51]. At this, the independent nature of this association is

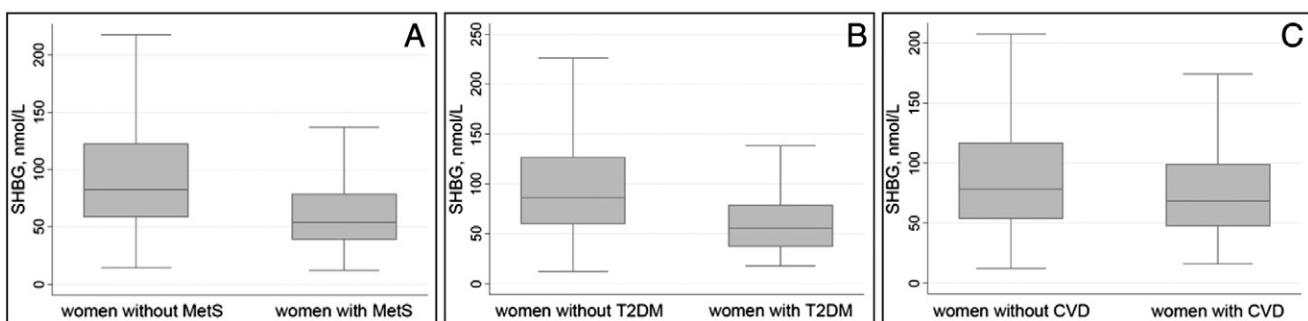


Fig. 3 – Associations between SHBG concentration and metabolic syndrome, type 2 diabetes mellitus, and cardiovascular disease. Box plots of associations between sex hormone-binding globulin (SHBG) and women with versus women without the following disease: A) metabolic syndrome (MetS), B) type 2 diabetes mellitus (T2DM) and C) cardiovascular disease (CVD). A) and B) are significant associations in age- and multivariable adjusted linear regression models. C) is a significant association only in age-adjusted models. Whiskers are defined as the most extreme values within the upper quartile + 1.5 (Q3–Q1) and the lower quartile – 1.5 (Q3–Q1), respectively.

still controversially discussed, since especially visceral obesity is well known as strong confounding factor [52,53]. However, in the present study, associations of MetS and T2DM with SHBG and fT were independent of behavioral, socio-demographic, and cardiometabolic correlates including adjustment for BMI. As for CVD, we only observed age-adjusted inverse associations with SHBG, ASD, and DHEAS. This finding is in line with previous studies, showing that SHBG is inversely associated with prevalent CVD only in age-adjusted models [18]. Taking underlying CVD risk factors into account, sex hormones were no longer associated with CVD [18]. This divergent finding might be explained by the comparatively healthy participants in our study sample and the low number of individuals with prevalent CVD. Additionally, the potential CVD risk of adverse sex hormone profiles might rather be linked to intermediate CVD risk factors, than to prevalent clinical CVD itself.

In summary, the present findings are in line with results from comparable population-based studies, including the Study of Women's Health Across the Nation (SWAN) [5,54], the Beaver Dam Eye Study [55], the Massachusetts cross-sectional study on health and aging [25,56] or the Women's Health Study (WHS) [57]. Despite varying age distributions, sample characteristics (SHIP: pre- and post-menopausal women, SWAN: pre-and peri-menopausal women, Beaver Dam Eye Study: post-menopausal women), hormone measurement methods, and blood sampling procedures (fasting vs. non-fasting blood samples), these studies show a strong overlap in their revealed findings. In general, androgenization was associated with an increased cardiovascular risk factor burden, with SHBG appearing as the strongest correlate of various cardiovascular risk factors. Additionally, the identified clinical correlates of sex hormones in women also show a relevant overlap across different ethnic groups (Caucasian, African-American, Hispanic, Chinese-American, and Japanese-American) and geographic regions [5,54,55].

In conclusion, we observed a number of associations between common CVD risk factors and sex hormones, suggesting that sex hormones are linked to an increased cardiometabolic risk factor burden.

4.1. Strengths and Limitations

The study has some limitations. Since validated data of self-reported PCOS are not collected in this study, we were not able to adjust for PCOS or exclude women with PCOS. The link between PCOS and decreased SHBG is due to effects of obesity and subsequently increased insulin responses [58]. Thus, despite the lack of PCOS data, we carefully adjusted for waist-circumference, involving the link between SHBG concentrations and obesity in all analyses. Additionally, blood samples for sex hormone measurements were taken during any phase of the menstrual cycle and we did not assess the phase of the menstrual cycle in SHIP-0. Consequently, we were not able to adjust for this potential source of bias. Since the present study was conducted in north-eastern Germany among white Caucasian women, the findings of this study are not generally applicable to other ethnic groups or geographic regions. Strengths of the present study include the use of LC-MS/MS-based measurements which improve hormone data especially in women as LC-MS/MS has the advantage to measure the low

concentrations range in women with high accuracy and sensitivity [59,60]. Furthermore, we analyzed a large study sample with adult women aged between 20 and 80 years from the general population with standardized data collection performed by trained and certified examiners.

4.2. Conclusion

The present cross-sectional study with highly sensitive assessment of androgen status in women based on LC-MS/MS, revealed associations of behavioral, socio-demographic, and clinical correlates including smoking, BMI, systolic blood pressure, LDL cholesterol, and MetS with endogenous sex hormones and SHBG. But despite this interplay between cardiometabolic risk factors and hormone metabolism, CVD itself was not independently associated with endogenous sex hormones in women. To reveal further correlates of sex hormones and SHBG, future clinical research with large randomized controlled clinical trials will be needed.

Disclosure Statement

The authors have nothing to disclose. All authors have approved the final article.

Funding and Acknowledgments

SHIP is part of the Community Medicine Research net (CMR) of the University of Greifswald, Germany, which is funded by the Federal Ministry of Education and Research (grants no. 01ZZ9603, 01ZZ0103, and 01ZZ0403), the Ministry of Cultural Affairs, as well as the Social Ministry of the Federal State of Mecklenburg-West Pomerania. This work is also part of the research project Greifswald Approach to Individualized Medicine (GANI_MED), and was supported by the DZHK (German Centre for Cardiovascular Research), and by the BMBF (German Ministry of Education and Research).

Appendix A. Supplementary Data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.metabol.2016.05.011>.

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Endogenous Androgens and Sex Hormone-Binding Globulin in Women and Risk of Metabolic Syndrome and Type 2 Diabetes

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Context and Objectives: The association of endogenous androgens and sex hormone-binding globulin (SHBG) with metabolic syndrome (MetS) and type 2 diabetes mellitus (T2DM) mostly refers to small and selected study samples with immunoassay-based measurements. Thus, we investigated the association of hormone levels with MetS and T2DM in women from a large population-based sample.

Design, Setting, and Participants: A total of 2077 women from the Study of Health in Pomerania were assessed at baseline (N = 3160, 1997–2001) and 5-year follow-up (N = 1711, 2002–2006).

Main Outcomes and Measures: We investigated associations of total T and androstenedione measured by liquid chromatography-tandem mass spectrometry, SHBG by immunoassay, and free T and free androgen index with MetS and T2DM.

Results: Baseline prevalence of MetS and T2DM was 23.1% (N = 365) and 9.5% (N = 196), with an incidence of 17.7 and 7.0 per 1,000 person-years, respectively. Cross-sectional analyses yielded inverse associations of SHBG with MetS (relative risk [RR], 0.67; 95% confidence interval [CI], 0.60–0.74) and T2DM (RR, 0.61; 95% CI, 0.50–0.74) after multivariable adjustment. In longitudinal analyses, only age-adjusted models showed an inverse association of baseline SHBG with incident MetS (RR, 0.61; 95% CI, 0.51–0.73) and T2DM (RR, 0.58; 95% CI, 0.43–0.78). Multivariable-adjusted models stratified by menopausal status revealed an inverse association between SHBG and incident MetS risk in postmenopausal women (RR, 0.65; 95% CI, 0.51–0.81).

Conclusions: This longitudinal population-based study revealed independent inverse associations of SHBG with MetS and T2DM, suggesting low SHBG as a potential risk marker for cardiometabolic morbidity, especially among postmenopausal women. (*J Clin Endocrinol Metab* 100: 0000–0000, 2015)

The number of type 2 diabetes mellitus (T2DM) patients worldwide is estimated to be 171 million and the number of incident cases is rising (1). A similar trend is

seen for metabolic syndrome (MetS), a multifactorial cluster of cardiometabolic risk factors including obesity, hypertriglyceridemia, hypertension, and insulin resistance

ISSN Print 0021-972X ISSN Online 1945-7197

Printed in USA

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Received June 11, 2015. Accepted October 1, 2015.

First Published Online October 7, 2015

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Abbreviations: ASD, androstenedione; ATC, Anatomical Therapeutic Chemical; BP, blood pressure; CI, confidence interval; FAI, free androgen index; fT, free T; IA, immunoassay; LC-MS/MS, liquid chromatography tandem mass spectrometry; MetS, metabolic syndrome; PCOS, polycystic ovary syndrome; Q, quartile; RR, relative risk; SHBG, sex hormone-binding globulin; T2DM, type 2 diabetes mellitus; TT, total T; WC, waist circumference.

(2), which can be detected in 20–25% of the global adult population (3). A potential link between low total T (TT) levels in men (4) and high TT levels in women with MetS has been suggested by observational research (5) and a recent meta-analysis of cross-sectional data (6). Furthermore, low sex hormone-binding globulin (SHBG) is associated with MetS development in both sexes (6). Especially in the subgroup of postmenopausal women, low SHBG levels were associated with all of the individual components of the MetS (7). T2DM typically depends on age and insulin resistance (8), with observational evidence that endogenous androgens and SHBG are also linked to T2DM (9). But although low levels of TT in men and high levels of bioavailable T in postmenopausal women increase the risk of T2DM, the association is not completely independent of adiposity, insulin resistance, or SHBG levels (10–13). Consequently, low SHBG was described as independent predictor of incident T2DM (10), reflecting a prediabetic state along with obesity and higher glucose and insulin levels (11, 14, 15).

However, these previous studies are limited either by their cross-sectional study design, selected samples, or immunoassay-based measurements of the androgens. The latter reason is an important limitation because all of the previously shown risk associations rely on immunoassay-based sex hormone measurements. Although immunologic androgen measurements are quite adequate for measuring T levels in healthy men, they must be cautiously evaluated in the low concentration range in women for reasons of insufficient sensitivity and accuracy (16–18). Consequently, this is the first population-based, longitudinal, observational study using liquid chromatography tandem mass spectrometry (LC-MS/MS)-based measurements for endogenous androgens to assess the potential associations between endogenous androgens and SHBG levels and cardiometabolic risk in women from the general population.

Materials and Methods

Study population

We used data from the Study of Health in Pomerania, a population-based cohort study in northeastern Germany. We previously published details of the study design, recruitment, and procedures (19). In brief, based on 6265 (3160 women) eligible individuals with German citizenship and main residency in the study area of Study of Health in Pomerania, 4308 individuals, aged 20–80 years, eventually were included between 1997 and 2001 in the baseline study (response, 68.8%). The study conformed to the principles of the Declaration of Helsinki and the protocol was approved by the Ethics Committee of the University of Greifswald. Written informed consent was received from each participant. Of the 2192 female baseline participants, we

excluded women with (overlap exists) pregnancy ($n = 18$) and women with self-reported bilateral oophorectomy or hysterectomy ($n = 52$). We excluded another 45 women who received sex hormone antagonists, natural opium alkaloids, and glucocorticoids, yielding a final study population of 2077 women. Although the intake of oral contraceptives in our study region is considerable (20), we did not exclude women currently taking oral contraceptives because this may have restricted the representativity of the study population, but we did perform sensitivity analyses. At the 5-year follow-up, 1711 female baseline participants (response, 76.6%) were reexamined between 2002 and 2006.

Measures

A computer-assisted personal interview served as a basis for information about sex, age, alcohol consumption, pregnancy, gynecological surgery (including bilateral oophorectomy and/or hysterectomy), and medication use. All prescribed drugs taken in the last 7 days were recorded based on the medication packages or, if not available, on self-statement and categorized based on the Anatomical Therapeutic Chemical (ATC) classification index. Women were defined as being physically inactive if they participated in physical training less than 1 hour a week. Smoking habits were evaluated by dividing women into three different categories: current, former, and never-smokers. For calculating mean daily alcohol consumption, beverage-specific pure ethanol volume proportions were used (21). A previously established definition from our cohort was used to categorize the population into pre- or postmenopausal women: premenopausal: all women younger than 40 years of age and between 40 and 60 years of age who reported a menstrual cycle; postmenopausal: all women at least 60 years of age and all women between 40 and 60 years who reported no menstrual cycle (22). Based on ATC codes, oral contraceptive (G03A) and hormone therapy (G03C, G03D, or G03F) were assessed (23). Waist circumference (WC) was measured to the nearest 0.1 cm using an inelastic tape midway between the lower rib margin and the iliac crest in the horizontal plane. After taking anthropometric measurements, body mass index was computed from body weight in kilograms and height in meters according to the formula: kg/m^2 .

Systolic and diastolic blood pressure (BP) were quantified three times on the right arm of seated subjects with an interval of 3 minutes between readings; the final two measurements were averaged. Measurement was performed after an initial resting period of at least 5 minutes by usage of an oscillometric digital BP monitor (HEM-705CP, Omron Corporation). Hypertension was defined as systolic or diastolic BP of higher than 140 mm Hg or lower than 90 mm Hg, respectively, or use of antihypertensive medication (ATC codes C02, C03, C04, C07, C08, C09) (24). T2DM was determined based on self-reported physician's diagnosis, use of antidiabetic medication (ATC code A10), and/or glycosylated insulin levels higher than 6.5% and lower than 20%. MetS components (bivariate variables) were defined based on 1) WC greater than 102 cm, 2) BP higher than 130/85 mm Hg or self-reported antihypertensive drug treatment, 3) nonfasting glucose at least 6.1 mmol/liter or antidiabetic treatment (ATC codes A10A, A10B), 4) nonfasting triglycerides at least 1.7 mmol/liter or lipid-lowering treatment (ATC codes C10AB, A10AD), and/or 5) HDL cholesterol no more than 1.3 mmol/liter. These five diagnostic criteria for the assessment of MetS are premised on the Joint Scientific Statement to harmonize MetS

(25) and further altered for the use of nonfasting blood samples (26). MetS was defined as having more than three components present. Both outcome variables (T2DM and MetS) were measured at baseline and follow-up.

We previously published a precise description of the measurements and age-specific reference ranges for levels of TT and androstenedione (ASD) (23). Summarizing, between 8:00 AM and 7:00 PM, a blood sample was drawn from the cubital vein in the supine position. Serum aliquots were stored at -80°C and carried out in a 4-month period (December 2010–March 2011) in the Department of Clinical Chemistry at the University Hospital of South Manchester (Manchester, UK). In Manchester, the frozen aliquots were used to conduct LC-MS/MS with a validated routine method (27). The standard curve was linear to 50.0 nmol/liter, the lower limit of quantitation was 0.25 nmol/liter, and intra- and interassay coefficients of variation were less than 10% for both TT and ASD over a range of 0.3–35 nmol/liter. The frozen serum aliquots were also used for measurements of SHBG levels using an Advia Centaur (Siemens) with an interassay coefficient of variation of 6.6% at the 27.1 nmol/liter level, 7.6% at the 48.2 nmol/liter level, and 7.7% at the 52.3 nmol/liter level. As previously described, free T (fT) was calculated as a relation between measured TT and SHBG levels (equations 1–4) for a standard average albumin concentration of 4.3 g/dl (28).

$$fT[\text{nmol/L}] = ((-a + \sqrt{b})/c)/10^{-9} \quad (1)$$

$$a = \text{SHBG}[\text{nmol/L}] - \text{TT}[\text{nmol/L}] + 23.43 \quad (2)$$

$$b = a^2 + (4 \cdot 23.43 \cdot \text{TT}[\text{nmol/L}]) \quad (3)$$

$$c = 2 \cdot 23.43 \cdot 10^9 \quad (4)$$

Free androgen index was calculated as a ratio of TT to SHBG (29). All hormone levels were measured at baseline only.

Statistical analysis

Categorical data are given as percentage; continuous data are given as mean (SD) or median (25th, 75th percentile). For group comparisons, the χ^2 test (categorical data) or the Mann-Whitney U test (continuous data) was used. We used the natural logarithmic transformation of the skewed continuous hormone variables to obtain normality. To examine cross-sectional and longitudinal associations of TT, SHBG, fT, ASD, and free androgen index (FAI) with MetS and T2DM as dependent variables, we implemented age- and multivariable-adjusted generalized Poisson-regression models with robust standard errors (30). Reported were relative risks (RR) and their 95% confidence intervals (95% CI) per SD increase in log hormones and for hormone quartiles with reference quartile one. The multivariable regression model included age, WC, smoking status, physical inactivity, and alcohol consumption. A P for trend test was performed by including the median of each sex hormone quartile as an ordinal score into the regression model. To estimate the relation of baseline TT, SHBG, fT, ASD, and FAI with prevalent and incident MetS or T2DM, hormone levels were categorized into 10-year age-specific quartiles. We performed longitudinal incidence analyses only in women without prevalent baseline MetS or T2DM, respectively. Multiplicative interaction terms between each hormone and menopausal status and use of oral contraceptives and hormone therapy were investigated using multivariable models. Models were retained when P values for interactions terms were <.05. We found significant interactions terms

for SHBG and FAI with tested covariates, thus we performed analyses separately in these groups. All models were stratified by menopausal status. A significance level of two-sided probability values <.05 was used in all analyses. This manuscript was written in accordance with the Strengthening the Reporting of Observational Studies in Epidemiology statement, giving guidelines for the reporting of observational studies (31). Stata 13.0 (Stata Corp) was applied for all statistical analyses.

Results

Table 1 presents the baseline characteristics for the full study sample and by menopausal status. Premenopausal women constituted 53.9% of the sample and showed a more favorable cardiovascular risk factor profile, including lower BP values and prevalence of hypertension, T2DM, and MetS, than postmenopausal women.

As shown in Tables 2 and 3, we detected an inverse association of SHBG with presence of both MetS (RR, 0.67; 95% CI, 0.60–0.74) and T2DM (RR, 0.61; 95% CI, 0.50–0.74) in multivariable cross-sectional analyses. Accordingly, multivariable adjusted RRs for SHBG levels in the highest compared to the lowest quartile were significant for MetS (quartile [Q1 [Ref.] vs Q4: RR, 0.45; 95% CI, 0.28–0.72) and T2DM (Q1 [Ref.] vs Q4: RR, 0.40; 95% CI, 0.24–0.68). The fT showed a positive association with MetS (RR, 1.20; 95% CI, 1.09–1.33) and with T2DM (RR, 1.30; 95% CI, 1.09–1.55).

Table 4 presents longitudinal Poisson-regression models and associations of baseline endogenous androgens and SHBG with incident MetS. Over the follow-up period (median, 5.04 years), the prevalence of MetS showed an increase from 365 women (23.1%) in the baseline sample to 413 (33.3%) in the follow-up sample. The MetS incidence per 1000 person-years was 17.7. Longitudinal analyses revealed an inverse association of SHBG with incident MetS (RR, 0.75; 95% CI, 0.63–0.89) in multivariable models. The fT level showed a positive association with incident MetS only in age-adjusted models (RR, 1.38; 95% CI, 1.16–1.65), but not in multivariable models (RR, 1.11; 95% CI: 0.95–1.30). P for trend analyses showed an inverse relationship across SHBG quartiles in all women in age- and multivariable-adjusted models. Multivariable Poisson-regression models revealed that quartile 3 (Q1 [Ref.] vs Q3: RR, 0.60; 95% CI, 0.40–0.89) and quartile 4 (Q1 [Ref.] vs Q4: RR, 0.50; 95% CI, 0.32–0.80) of SHBG levels were inversely associated with incident MetS in all women. Analyses stratified by menopausal status (Supplemental Table 1) revealed an inverse association between SHBG and incident MetS in postmenopausal women in multivariable-adjusted models (RR, 0.65; 95% CI, 0.51–0.81); Q1 [Ref.] vs Q4: RR, 0.39; 95% CI, 0.20–0.77). Cross-sectional analyses revealed a significant trend

Table 1. Baseline Characteristics of the Study Population

Variable	All Women (n = 2077)	Premenopausal Women (n = 1111)	Postmenopausal Women (n = 963)
Age, years	48.8 (16.2)	36.0 (9.6)	63.0 (9.2)
BMI, kg/m ²	26.1 (5.4)	24.0 (5.0)	28.3 (5.2)
WC, cm	81.5 (13.2)	75.8 (12.0)	87.5 (12.2)
Serum total T, nmol/liter	0.76 (0.55, 1.07)	0.83 (0.60, 1.14)	0.68 (0.50, 0.97)
Serum SHBG, nmol/liter	82.8 (57.8, 123.5)	100.4 (67.2, 154.1)	71.6 (49.9, 96.5)
Serum free T, nmol/liter	0.007 (0.004, 0.011)	0.006 (0.004, 0.010)	0.007 (0.005, 0.011)
Serum ASD, pmol/liter	1.61 (1.05, 2.51)	2.24 (1.49, 3.18)	1.18 (0.86, 1.62)
Free androgen index	0.90 (0.55, 1.52)	0.82 (0.48, 1.41)	0.99 (0.62, 1.62)
Current smoking, %	27.1	36.8	16.0
Alcohol consumption, g/day	2.2 (0.0, 7.5)	2.5 (0.0, 8.7)	0.0 (0.0, 5.0)
Physically inactive, %	56.8	48.1	66.7
Oral contraceptive use, %	17.7	33.0	0.0
Hormone therapy, %	9.0	6.4	12.1
Systolic BP, mm Hg	127.3 (21.5)	118.7 (16.0)	140.0 (21.7)
Diastolic BP, mm Hg	80.7 (10.7)	78.0 (10.1)	83.7 (10.8)
Antihypertensive medication, %	19.9	4.3	41.7
Hypertension, %	38.6	25.7	53.4
T2DM, %	9.5	1.4	18.9
MetS, %	23.1	8.1	37.4

Data are percentages, mean (sd), or median (Q1, Q3).

Abbreviations: ASD, androstenedione; BMI, body mass index; BP, blood pressure; MetS, metabolic syndrome; Q, quarter; SHBG, sex hormone-binding globulin; T2DM, type 2 diabetes mellitus; WC, waist circumference.

Oral contraceptive use was defined according to Anatomical Therapeutic Chemical classification code G03AA/B/C/D. Values of these hormones are reported based on availability of each hormone. Serum total T, n = 1598; SHBG, n = 1925; serum androstenedione, n = 1655; serum free T, n = 1491; free androgen index, n = 1491.

of lower SHBG and ASD ($P < .01$), but not TT levels with increasing numbers of MetS components at baseline (Figure 1).

Table 5 presents longitudinal age-adjusted Poisson-regression models and shows associations of baseline endogenous androgens and SHBG with incident T2DM.

Over the follow-up period, the prevalence of T2DM showed only a slight increase from 9.5% in the baseline sample to 10.9% in the follow-up sample. The incidence of T2DM per 1000 person-years was 7.0. Longitudinal models showed also an inverse association between SHBG with incident T2DM (RR, 0.58; 95% CI, 0.43–0.78), but

Table 2. Cross-Sectional Association of Baseline Endogenous Androgens and SHBG With MetS in Women

	RR (95% CI)				
	Total T	SHBF	Free T	ASD	Free Androgen Index
All Women, age-adjusted models					
Contin.	0.96 (0.88, 1.05)	0.53 (0.48, 0.58) ^a	1.40 (1.26, 1.54) ^a	0.99 (0.88, 1.11)	1.52 (1.37, 1.68) ^a
<25th percentile	Ref.	Ref.	Ref.	Ref.	Ref.
25th–50th percentile	1.02 (0.78, 1.34)	0.55 (0.45, 0.67) ^a	1.64 (1.17, 2.30) ^a	0.94 (0.72, 1.21)	1.64 (1.15, 2.34) ^a
50th–75th percentile	1.07 (0.82, 1.40)	0.34 (0.26, 0.44) ^a	1.96 (1.40, 2.73) ^a	1.05 (0.81, 1.35)	2.04 (1.45, 2.87) ^a
>75th percentile	0.99 (0.76, 1.31)	0.25 (0.18, 0.34) ^a	2.46 (1.80, 3.37) ^a	1.03 (0.79, 1.34)	2.89 (2.09, 4.00) ^a
P for trend	.99	<.01	<.01	.66	<.01
All women, multivariable-adjusted models					
Contin.	0.97 (0.89, 1.05)	0.67 (0.60, 0.74) ^a	1.20 (1.09, 1.33) ^a	0.95 (0.84, 1.06)	1.26 (1.14, 1.40) ^a
<25th percentile	Ref.	Ref.	Ref.	Ref.	Ref.
25th–50th percentile	0.84 (0.66, 1.08)	0.68 (0.56, 0.82) ^a	1.27 (0.92, 1.76)	0.91 (0.72, 1.14)	1.19 (0.84, 1.68)
50th–75th percentile	1.01 (0.79, 1.29)	0.48 (0.37, 0.62) ^a	1.43 (1.05, 1.95) ^a	1.07 (0.84, 1.36)	1.42 (1.03, 1.97) ^a
>75th percentile	0.89 (0.68, 1.15)	0.45 (0.28, 0.72) ^a	1.56 (1.15, 2.11) ^a	0.90 (0.70, 1.15)	1.69 (1.23, 2.34) ^a
P for trend	0.33	<0.01	<0.01	0.33	<0.01

Abbreviations: ASD, androstenedione; BMI, body mass index; BP, blood pressure; CI, confidence interval; contin., continuous; MetS, metabolic syndrome; Q, quarter; RR, relative risk; Ref., reference; SHBG, sex hormone-binding globulin; T2DM, type 2 diabetes mellitus; WC, waist circumference.

The multivariable model was adjusted for age, WC, smoking status (three categories), physical inactivity, and alcohol consumption. The presented data show the relative risk (95% CI); ^a $P < .05$. Values of these hormones are reported based on availability of each hormone. Serum total T, n = 1598; SHBG, n = 1925; serum androstenedione, n = 1655; serum free T, n = 1491; free androgen index, n = 1491.

Table 3. Cross-Sectional Association of Baseline Endogenous Androgens and SHBG with T2DM in Women

	RR (95% CI)				
	Total T	SHBG	Free T	ASD	Free Androgen Index
All women, age-adjusted models					
Contin.	0.93 (0.81, 1.07)	0.52 (0.43, 0.62) ^a	1.44 (1.21, 1.70) ^a	1.01 (0.85, 1.21)	1.61 (1.35, 1.92) ^a
<25th percentile	Ref.	Ref.	Ref.	Ref.	Ref.
25th–50th percentile	0.77 (0.51, 1.15)	0.69 (0.50, 0.96) ^a	1.16 (0.69, 1.96)	0.83 (0.55, 1.24)	1.17 (0.68, 2.01)
50th–75th percentile	0.63 (0.41, 0.98) ^a	0.42 (0.29, 0.63) ^a	1.43 (0.86, 2.36)	0.97 (0.65, 1.43)	1.57 (0.94, 2.62) ^a
>75th percentile	0.87 (0.60, 1.27)	0.28 (0.18, 0.45) ^a	2.20 (1.42, 3.42) ^a	0.93 (0.62, 1.37)	2.65 (1.68, 4.18) ^a
P for trend	.59	<.01	<.01	.92	<.01
All women, multivariable-adjusted models					
Contin.	0.94 (0.82, 1.09)	0.61 (0.50, 0.74) ^a	1.30 (1.09, 1.55) ^a	1.00 (0.83, 1.19)	1.42 (1.19, 1.70) ^a
<25th percentile	Ref.	Ref.	Ref.	Ref.	Ref.
25th–50th percentile	0.66 (0.44, 0.99) ^a	0.79 (0.57, 1.10)	1.00 (0.59, 1.69)	0.85 (0.57, 1.28)	0.93 (0.53, 1.60) ^a
50th–75th percentile	0.63 (0.42, 0.94) ^a	0.52 (0.35, 0.78) ^a	1.27 (0.77, 2.12)	1.04 (0.70, 1.54)	1.32 (0.79, 2.21)
>75th percentile	0.81 (0.55, 1.19)	0.40 (0.24, 0.68) ^a	1.68 (1.07, 2.62) ^a	0.89 (0.60, 1.31)	1.87 (1.17, 3.01)
P for trend	.46	<.01	<.01	.66	<.01

Abbreviations: ASD, androstenedione; BMI, body mass index; BP, blood pressure; CI, confidence interval; contin., continuous; MetS, metabolic syndrome; Q, quarter; RR, relative risk; Ref., reference; SHBG, sex hormone-binding globulin; T2DM, type 2 diabetes mellitus; WC, waist circumference.

The multivariable model was adjusted for age, waist circumference, smoking status (three categories), physical inactivity, and alcohol consumption. The presented data show the relative risk (95% CI); ^a P < 0.05. Values of these hormones are reported based on availability of each hormone. Serum total T, n = 1598; SHBG, n = 1925; serum ASD, n = 1655; serum free T, n = 1491; free androgen index, n = 1491.

in contrast to incident MetS only in the age-adjusted model (multivariable model: RR, 0.88; 95% CI, 0.65–1.19). The fT level revealed similar associations with incident T2DM as with incident MetS (age-adjusted model: RR, 1.37; 95% CI, 1.07–1.77; multivariable model: RR, 1.06; 95% CI, 0.79–1.42) (Table 5). Finally, stepwise multivariable regression analyses revealed that WC was the most relevant confounder for the attenuation in significance level

between the age-adjusted model and the fully adjusted model (Supplemental Table 2). Analyses stratified by menopausal status did not yield any significant association of endogenous androgens and SHBG and T2DM in pre- or postmenopausal women after multivariable adjustment (Supplemental Table 1). The exclusion of women using oral contraceptives or hormone therapy in cross-sectional and longitudinal analyses did not substantially

Table 4. Longitudinal Association of Baseline Endogenous Androgens and SHBG With Incident MetS in Women

	RR (95% CI)				
	Total T	SHBG	Free T	Androstenedione	Free Androgen Index
All women, age-adjusted models					
Contin.	0.97 (0.84, 1.12)	0.61 (0.51, 0.73) ^a	1.32 (1.11, 1.56) ^a	1.12 (0.94, 1.34)	1.38 (1.16, 1.65) ^a
<25th percentile	Ref.	Ref.	Ref.	Ref.	Ref.
25th–50th percentile	1.37 (0.89, 2.11)	0.70 (0.49, 0.99) ^a	0.45 (0.89, 2.35)	1.27 (0.81, 1.98)	1.18 (0.71, 1.97)
50th–75th percentile	1.17 (0.73, 2.11)	0.48 (0.32, 0.71) ^a	1.34 (0.80, 2.23)	1.38 (0.89, 2.14)	1.48 (0.92, 2.39)
>75th percentile	0.97 (0.59, 1.60)	0.30 (0.19, 0.47) ^a	2.23 (1.41, 3.53) ^a	1.25 (0.78, 1.99)	2.25 (1.43, 3.53) ^a
P for trend	.86	<.01	<.01	.37	<.01
All women, multivariable-adjusted models					
Contin.	0.94 (0.82, 1.06)	0.75 (0.63, 0.89) ^a	1.11 (0.95, 1.30)	1.02 (0.87, 1.20)	1.14 (0.97, 1.35)
<25th percentile	Ref.	Ref.	Ref.	Ref.	Ref.
25th–50th percentile	1.08 (0.71, 1.63)	0.93 (0.68, 1.29)	1.31 (0.81, 2.09)	1.14 (0.75, 1.72)	1.17 (0.71, 1.93)
50th–75th percentile	1.88 (0.55, 1.39)	0.60 (0.40, 0.89) ^a	1.15 (0.71, 1.88)	1.10 (0.71, 1.69)	1.30 (0.82, 2.06)
>75th percentile	0.84 (0.55, 1.29)	0.50 (0.32, 0.80) ^a	1.34 (0.85, 2.13)	1.09 (0.71, 1.66)	1.35 (0.85, 2.14)
P for trend	.27	<.01	.30	.78	.20

Abbreviations: ASD, androstenedione; BMI, body mass index; BP, blood pressure; CI, confidence interval; contin., continuous; MetS, metabolic syndrome; Q, quarter; RR, relative risk; Ref., reference; SHBG, sex hormone-binding globulin; T2DM, type 2 diabetes mellitus; WC, waist circumference.

Results stratified by menopausal status are provided in Supplemental Table 1. The sample was limited to women without baseline MetS. The multivariable model was adjusted for age, waist circumference, smoking status (three categories), physical inactivity, and alcohol consumption. The presented data show the relative risk (95% CI); ^a P < .05. Values of these hormones are reported based on availability of each hormone. Serum total T, n = 1598; SHBG, n = 1925; serum androstenedione, n = 1655; serum free T, n = 1491; free androgen index, n = 1491.

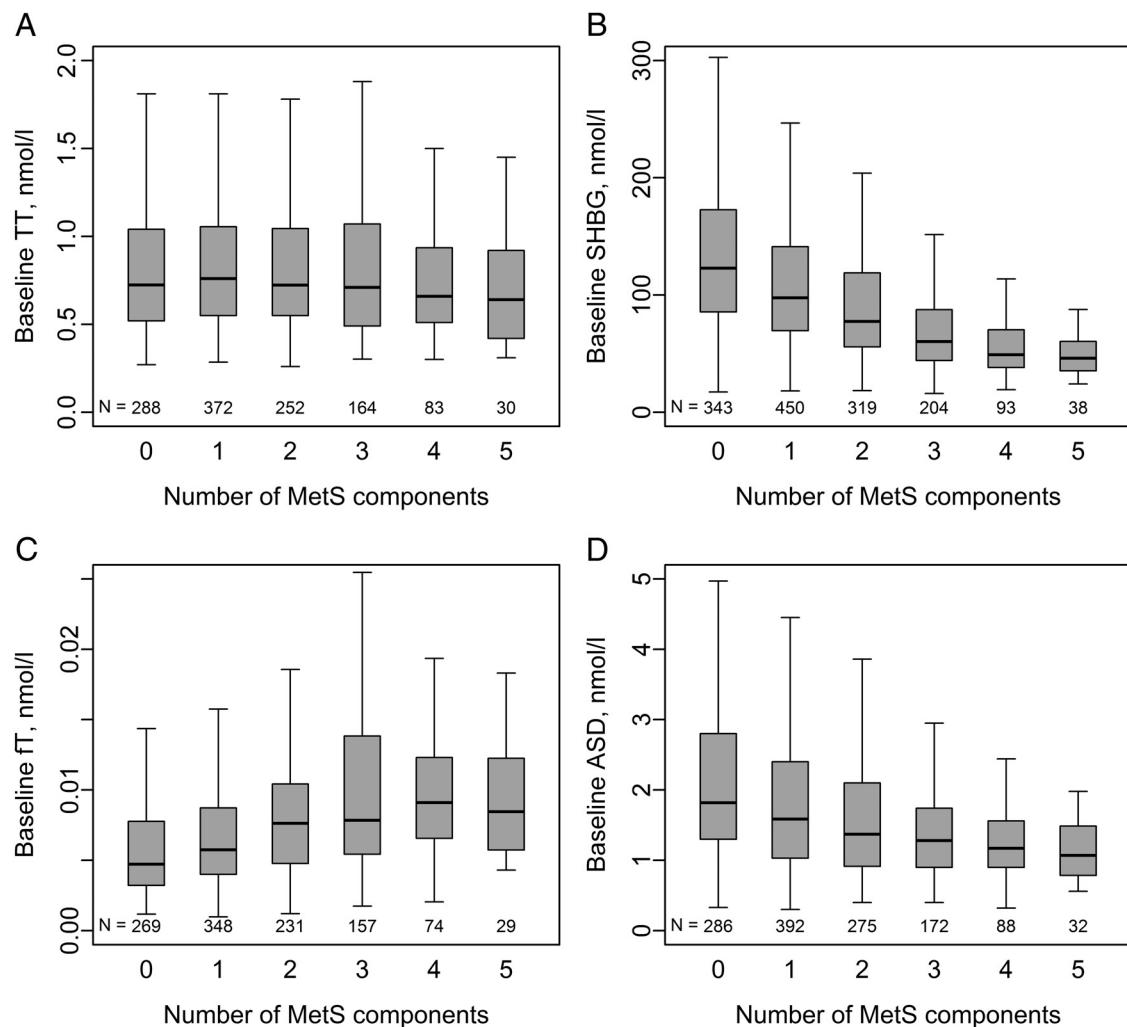


Figure 1. Boxplots for endogenous androgens and sex hormone–binding globulin (SHBG) according to number of metabolic syndrome components at baseline. Means with 95% confidence interval in the analytical sample ($n = 2077$) for A) total T (TT), B) SHBG, C) free T (fT), and D) androstenedione (ASD) levels according to zero, one, two, three, four, or five components of metabolic syndrome (MetS) at baseline.

alter the revealed estimates for both outcomes (data not shown).

Discussion

The present longitudinal, population-based study is the first one that investigated an association of LC-MS/MS measured endogenous androgens and SHBG with MetS and T2DM in women. We were able to demonstrate a statistically significant association between low SHBG levels and an increased MetS risk after multivariable adjustment. Although TT showed no associations with incident MetS or T2DM, low SHBG and therefore high fT levels were associated with incident T2DM in age-adjusted analyses.

In light of the present findings, previously published studies reported similarly conflicting results, especially with regard to the influence of TT and fT on MetS risk. For example, a cross-sectional population-based study among

212 postmenopausal women yielded a strong association between low SHBG and prevalent MetS (7). A meta-analysis of 19 observational studies including 7839 women showed an association of TT, fT, and SHBG with incident MetS (6). However, all cited studies are limited by the measurement of sex hormones by immunoassays.

Interestingly, the longitudinal Study of Women's Health Across the Nation among 1862 pre- and postmenopausal women suggested the ratio between T and estradiol as a stronger predictor of incident MetS than levels of specific sex hormone alone (32). Our study did not analyze sex hormone ratios because levels of estradiol or estrone were not measured. Because our sex hormone measurements are based on blood samples taken during any phase of the menstrual cycle and we did not assess the phase of the menstrual cycle, the interpretation of estrone and estradiol data would be questionable anyway. However, because sex hormone ratios are not sufficiently ex-

Table 5. Longitudinal Association of Baseline Endogenous Androgens and SHBG With Incident T2DM in Women

	RR (95% CI)				
	Total T	SHBG	Free T	ASD	Free Androgen Index
All women, age-adjusted models					
Contin.	0.96 (0.76, 1.20)	0.58 (0.43, 0.78) ^a	1.37 (1.07, 1.77) ^a	1.03 (0.83, 1.55)	1.47 (1.13, 1.90) ^a
<25th percentile	Ref.	Ref.	Ref.	Ref.	Ref.
25th–50th percentile	1.89 (0.85, 4.20)	0.63 (0.33, 1.18)	1.89 (0.72, 4.99)	1.46 (0.74, 2.88)	1.42 (0.55, 3.67)
50th–75th percentile	1.83 (0.84, 3.98)	0.40 (0.19, 0.86) ^a	2.39 (0.95, 6.02)	0.61 (0.25, 1.48)	1.97 (0.82, 4.72)
>75th percentile	1.04 (0.42, 2.57)	0.37 (0.18, 0.75) ^a	2.94 (1.17, 7.39) ^a	1.21 (0.59, 2.50)	1.67 (1.13, 6.29) ^a
P for trend	.70	<.01	.01	.16	.01
All women, multivariable-Adjusted Models					
Contin.	0.96 (0.75, 1.22)	0.88 (0.65, 1.19)	1.06 (0.79, 1.42)	1.12 (0.80, 1.58)	1.06 (0.79, 1.43)
<25th percentile	Ref.	Ref.	Ref.	Ref.	Ref.
25th–50th percentile	1.45 (0.66, 3.15)	0.89 (0.47, 1.68)	1.17 (0.44, 3.10)	1.06 (0.54, 2.09)	0.76 (0.29, 2.00)
50th–75th percentile	1.66 (0.75, 3.66)	0.71 (0.33, 1.52)	1.39 (0.57, 3.41)	0.58 (0.25, 1.35)	1.03 (0.44, 2.39)
>75th percentile	0.89 (0.36, 2.21)	0.90 (0.33, 1.52)	1.40 (0.53, 3.53)	0.96 (0.47, 2.00)	0.99 (0.40, 2.48)
P for trend	.56	.70	.53	.79	.71

Abbreviations: ASD, androstenedione; BMI, body mass index; BP, blood pressure; CI, confidence interval; contin., continuous; MetS, metabolic syndrome; Q, quarter; RR, relative risk; Ref., reference; SHBG, sex hormone-binding globulin; T2DM, type 2 diabetes mellitus; WC, waist circumference.

Results stratified by menopausal status are provided in Supplemental Table 1. The sample was limited to women without baseline T2DM. The multivariable model was adjusted for age, WC, smoking status (three categories), physical inactivity, and alcohol consumption. The presented data show the RR (95% CI); ^a P < .05. Values of these hormones are reported based on availability of each hormone. Serum total T, n = 1598; SHBG, n = 1925; serum androstenedione, n = 1655; serum free T, n = 1491; free androgen index, n = 1491.

amined to date, further research is indicated to examine the complex interplay of T/estrogen ratio and its potential influence on both MetS and T2DM.

Investigating the potential influence of endogenous androgens and SHBG on T2DM risk in women, previous studies showed conflicting results as well. For example, a meta-analysis of 36 cross-sectional studies comprising 4795 women showed that participants with high TT and low SHBG levels had a higher rate of incident T2DM concurrently (13). In contrast, the prospective Multi-Ethnic Study of Atherosclerosis study among 1612 postmenopausal women showed that SHBG was inversely associated with T2DM, whereas bioavailable T showed no association with incident T2DM after adjustment for body mass index and insulin resistance (12). These contrary results reflect the findings of a review among postmenopausal women, where most of the compiled studies did not show an association between TT and T2DM (14). A recent meta-analysis suggested that the association between SHBG and incident T2DM is independent (14), although a multiethnic study of 1612 healthy postmenopausal women suggested that it may partially be explained by adiposity and insulin resistance (12). Because the present significant association between SHBG and T2DM was retained after adjustment for smoking status, physical inactivity, and alcohol consumption, but cannot be confirmed after adjusting for WC, our findings support an association that is dependent on body composition and therefore not independent of especially visceral obesity. However, a

recent Mendelian randomization study supports a causal role of SHBG in insulin and glucose metabolism (14). On the contrary, prediabetic hyperinsulinemia is thought to decrease SHBG production, which suggests reverse causation, which is the reason we analyzed prospective data of women without baseline T2DM (33).

Facing the limits of observational research, future clinical studies are needed to elucidate the mechanism of cause or consequence in the association between SHBG and T2DM.

To decipher the cardiometabolic risk of androgens in women, further research should particularly focus not only on general population, but also should include special patients such as women with polycystic ovary syndrome (PCOS) to explain potential mechanisms of these divergent findings and further investigate whether endogenous androgens and SHBG might be risk markers for MetS or T2DM compared to men.

To explain the previously mentioned conflicting results of previous studies, it is necessary to consider several strengths and potential limitations. A major limitation in all of the previous studies is the hormone measurement based on immunoassay (IA) increasing measurement error and thereby diluting potential risk associations (5, 7, 12–14, 32). IA is not reliable for measuring low T levels as found in women (16, 17, 18). Furthermore, direct IAs are limited by overestimated T concentration, matrix effects, and cross-reactivity (34). Although there are labor-intensive assays that are more accurate and sensitive than direct

assays, there is no absolute selectivity for measuring T and they still require proper validation (18). In contrast, the current highly sensitive method based on LC-MS/MS allows accurate measurements of endogenous androgens, especially in women (17). The present study shows that endogenous androgens measurements based on LC-MS/MS does not improve risk prediction for MetS and T2DM vs IA measurements in the general population. Information in special risk groups such as women with PCOS are lacking to date. Nevertheless, MS offers the potential for standardization of endogenous androgens measurements, as recommended by the Endocrine Society and the International Federation of Clinical Chemistry and Laboratory Medicine (35, 36).

Additionally, the divergent findings might result from heterogeneous study designs, with most of the previous studies investigating only cross-sectional samples, whereas the potential effect of endogenous androgens and SHBG as risk markers for MetS and T2DM over a certain period was investigated only rarely (5, 7, 13, 14). Furthermore, certain studies are also limited by small study sample sizes (5, 7). Because the present study included more than 2000 women over a 5-year follow-up, this analysis represents the largest investigation of cardiometabolic risk of endogenous androgens and SHBG in women to date. Furthermore, the present study is based on a representative sample of the general population in Pomerania, whereas previous studies included selected participants, such as postmenopausal women (7, 12, 14). An additional strength of the current study is the standardized data collection performed by trained and certified examiners. Based on the knowledge that the prevalence of T2DM and MetS increases with age, it may be a reasonable explanation that the present study, with a median of 49 years, includes a low number of incident T2DM and MetS cases compared to previous studies. This comparably young and healthy study sample from the general population leads to a lower number of incident cases and thus lack of statistical power to detect potential risk associations.

Given that oral intake of estrogen increases levels of SHBG serum levels (15) and thereby decreases levels of endogenous androgens, it has to be mentioned that the intake of an oral contraceptive in our study region (20) and furthermore in East Germany (37) is considerable. Hence, we performed sensitivity analyses by including the stratification for oral contraceptive use and hormone replacement therapy.

The present findings may be limited by PCOS. The diagnostic criteria of the Rotterdam or the National Institutes of Health classification were not assessed in our cohort. In women with PCOS, SHBG levels are typically decreased because of the effects of obesity and following

increased insulin responses to decrease hepatic production of SHBG, thereby increasing androgen bioavailability (38). Finally, geographic, environmental, and ethnic discrepancies between the different study populations may result in variable risk associations between endogenous androgens and SHBG and cardiometabolic outcomes.

Conclusion and Perspectives

The present longitudinal population-based study revealed an inverse association of SHBG levels in women with MetS after multivariable adjustment and T2DM after age adjustment. Low SHBG levels might represent a risk marker for MetS as well as T2DM in women. In contrast to previous findings, we were not able to detect an association of TT to T2DM and MetS in the general population. To further illuminate the association between SHBG and sex hormones with incident MetS and T2DM in women, meta-analysis are needed, especially in specific risk conditions like PCOS. Future investigations should also apply harmonized LC-MS/MS measurements such as those recommended by the Task Force of the Endocrine Society (35).

Acknowledgments

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The Study of Health in Pomerania is part of the Community Medicine Research (CMR) net of the University of Greifswald, Germany, which is funded by the Federal Ministry of Education and Research (Grants 01ZZ9603, 01ZZ0103, and 01ZZ0403), the Ministry of Cultural Affairs, as well as the Social Ministry of the Federal State of Mecklenburg-West Pomerania. This work is also part of the research project Greifswald Approach to Individualized Medicine, and was supported by the DFG (German Research Foundation), the DZHK (German Centre for Cardiovascular Research), and the BMBF (German Ministry of Education and Research).

Disclosure Summary: The authors have nothing to disclose.

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Serum androgen concentrations and subclinical measures of cardiovascular disease in men and women



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ARTICLE INFO

Article history:

Received 14 December 2015

Received in revised form

9 February 2016

Accepted 17 February 2016

Available online 22 February 2016

Keywords:

Androgens

Sex hormone-binding globulin

Subclinical cardiovascular disease

Epidemiology

ABSTRACT

Objectives: Most of the observed associations of androgens and sex hormone-binding globulin (SHBG) with subclinical cardiovascular disease (CVD) stem from selected study samples with immunoassay-based hormone measurements. Thus, we used a large population-based sample with total testosterone (TT) and androstenedione (ASD) concentrations measured by liquid chromatography-tandem mass spectrometry.

Design: Data of 2140 individuals (mean age: 60.8 years) from the cohort Study of Health in Pomerania were assessed at baseline and 5-year follow-up.

Methods: Multivariable regression models were implemented to assess cross-sectional and longitudinal associations of TT, free testosterone (FT), ASD, SHBG and dehydroepiandrosterone-sulphate (DHEAS) with measures of subclinical CVD including intima media thickness (IMT), carotid plaques, left ventricular mass (LVM), fractional shortening (FS), relative wall thickness (RWT), and left ventricular geometry.

Results: Cross-sectional analyses yielded an association of TT with IMT in women (β -coefficient per log unit increase: 0.02; 95% CI: 0.007; 0.45) and ASD with FS in both sexes (men: β -coefficient: -2.94; 95% CI: -4.75; -1.12; women: β -coefficient: 1.64; 95% CI: 0.55; 2.73). In longitudinal analyses, DHEAS was positively associated with FS change (β -coefficient: 2.34; 95% CI: -0.59; 4.08). In women, SHBG was positively associated with incident plaques (Q1 vs. Q3 (Ref.): β -coefficient: 1.35; 95% CI: 1.04; 1.74). In both sexes, longitudinal analyses showed no consistent association of TT with subclinical CVD.

Conclusions: Despite several sex-specific associations of androgens and SHBG with subclinical CVD, the present representative study for the age group ≥ 45 years among men and women from the general population detected no consistent associations in longitudinal analyses.

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1. Introduction

Cardiac remodeling describes a physiological process of incremental alterations in the heart's structure and function over the life course [1], characterized by changes in several echocardiographic indices including changes in left ventricular mass (LVM), LV wall thickening, and geometrical LV adaptations. Contemporary

echocardiography and ultrasound techniques allow an early and non-invasive assessment of these subclinical measures of cardiovascular disease (CVD), including left ventricular hypertrophy (LVH), a structural adaptation of the heart to an increased blood pressure [2], changes in carotid intima media thickness (IMT), and modifications on the mesenchyme cells in vessel walls, called plaques [3]. Observational research provides solid evidence for a strong link of these measures with CVD onset and progression, as well as an increased mortality risk [4]. The potential link between androgens and subclinical CVD refers to animal models and observational research [3,4], suggesting sex-specific differences in CVD onset and progression [5]. Previous observational studies

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among men showed that total testosterone (TT), free testosterone (fT), dehydroepiandrosterone-sulphate (DHEAS), and sex hormone-binding globulin (SHBG) concentrations are inversely associated with carotid plaques, LVM [6,7] and IMT, partially mediated by obesity [3,7,8]. Studies among women reported that TT and fT were positively and SHBG inversely associated with IMT [3,9], whereas menopausal women showed no association of TT with IMT progression or subclinical atherosclerosis [10].

However, population-based analyses of longitudinal associations between endogenous androgens and SHBG and change in subclinical CVD among both sexes are scarce. Particularly immunoassay-based measurements of androgens are a common limiting factor in previous studies, especially among women [11]. Thus, the aim of the present study was to investigate cross-sectional and longitudinal associations between androgen and SHBG concentrations measured by liquid chromatography mass-spectrometry (LC-MS) and subclinical CVD in middle-aged men and women from a population-based cohort.

2. Methods

2.1. Study population

The population-based Study of Health in Pomerania (SHIP) is a cohort study, established in West-Pomerania, a region in north-eastern Germany. Details of the study design, procedures, and recruitment were published previously [12]. The target population was 6265 eligible individuals with German citizenship and main residency in the study area, selected from the population registration offices. 4308 individuals (2192 women) aged 20–80 years lastly participated between 1997 and 2001 in the baseline study (SHIP-0, response rate: 68.8%), after written informed consent was obtained from each participant. Five-year follow-up examinations were conducted between 2002 and 2006 (SHIP-1), involving 3300 individuals (1711 women, total response rate: 83.6%). The study was reviewed by the local ethical committee of the University Greifswald and is consistent with the principles of the Declaration of Helsinki. Echocardiography was performed in individuals aged 45 years or older. From these 2550 participants, 410 were excluded due to (overlap exists): self-reported hysterectomy ($N = 31$ women) or bilateral oophorectomy, medication use in the last seven days (based on the Anatomical Therapeutic Chemical classification index) including sex hormone antagonists ($N = 2$ women, 135 men), antiandrogens ($N = 7$ women), and natural opium alkaloids ($N = 23$ women, 14 men). Furthermore, we excluded participants with prevalent or incident myocardial infarction ($N = 45$ women, 107 men) and aortic stenosis ($N = 20$ women, 25 men). None of the women were pregnant at baseline or follow-up. Altogether, the final baseline study population comprised 1145 women and 995 men (see flow chart in Supplementary Fig. 1).

2.2. Measures

Information on sociodemographic and behavioral characteristics, as well as medical history, including information about sex, age, physical inactivity, smoking habits, pregnancy, medical procedures, and medication use were collected, using a computer-assisted personal interview. As to smoking habits, participants were divided into three categories (current, former, and never-smokers). Women were stratified into pre- and post-menopausal, using a previously published categorization [13]. The use of hormone therapy (G03C, G03D, or G03F) and oral contraceptive (G03A) was assessed based on ATC codes. Weight was measured utilizing standard digital scales (to the nearest 0.1 kg). Body mass index

(BMI) was calculated from the body weight in kilogram and height in meters [$BMI = \text{kg}/\text{m}^2$]. Systolic (SBP) and diastolic blood pressure (DBP) were measured after a resting period of at least five minutes and an interval between the three readings of three minutes, using an oscillometric digital blood pressure monitor (HEM-705CP, Omron Corporation, Tokyo, Japan). For the present analyses the mean of the second and third measurements was used. Hypertension was defined as use of antihypertensive medication or systolic blood pressure of at least 140 mm Hg or diastolic blood pressure of at least 90 mm Hg. Diabetes was defined as $\text{HbA1c} \geq 6.5$, glucose $\geq 11.1 \text{ mmol/L}$ or self-reported use of insulin or oral antidiabetic medications.

All hormone concentrations were measured at baseline and were previously described in detail [14]. Briefly, between 8:00 a.m. and 7:00 p.m. non-fasting serum samples were taken and stored at -80°C until measurement by LC-MS [15]. The lower limit of quantitation was 0.25 nmol/L and intra- and inter-assay coefficients of variation were <10% for both TT and ASD over the range 0.3–35 nmol/L. SHBG concentrations were measured from frozen serum aliquots using a chemiluminescent enzyme immunoassay Advia Centaur (Siemens, Eschborn, Germany) with an inter-assay coefficient of variation of 6.6% at the 27.1 nmol/L level, 7.6% at the 48.2 nmol/L level, and 7.7% at the 52.3 nmol/L level. DHEAS was measured only in men, using a competitive chemiluminescent enzyme immunoassay on an Immulite 2500 analyzer (DPC Biermann GmbH, Bad Nauheim, Germany). fT was calculated from measured TT and SHBG concentrations for a standard average albumin concentration of 4.3 g/dL (equations (1)–(4)) [16].

$$fT[\text{nmol/L}] = ((-a + \sqrt{b})/c)/10^{-9} \quad (1)$$

$$a = \text{SHBG}[\text{nmol/L}] - \text{TT}[\text{nmol/L}] + 23.43 \quad (2)$$

$$b = a^2 + (4 \cdot 23.43 \cdot \text{TT}[\text{nmol/L}]) \quad (3)$$

$$c = 2 \cdot 23.43 \cdot 10^9 \quad (4)$$

Carotid far-wall IMT scans were digitized through the axis of the distal straight portion (1 cm in length) of both common carotid arteries and recorded for subsequent offline analysis. Mean IMT was calculated by averaging the 10 consecutive measurement points (in 1 mm steps) from both sides. IMT was defined as the distance between the characteristic echoes from the lumen-intima and media-adventitia interfaces. Plaques were determined with sonography of the carotid artery. Echocardiography (two-dimensional and M-mode) was performed by certified physicians using a Vingmed CFM800A system (GE Medical Systems, Waukesha, Wisconsin, USA). M-mode images of the left ventricle were recorded at the papillary level. The leading-edge convention was used to measure left ventricular dimensions (posterior wall thickness (LVPWD), interventricular septum thickness (IVS), left ventricular end-diastolic (LVDD), and systolic (LVDS) diameter). LVM was calculated according to the following formula: $LVM = 0.80 \times (1.04 \times ([LVDD + IVS + LVPWD]^3 - LVDD^3]) + 0.60/\text{height}^{2.7}$. LVH was defined by a $LVM > 44 \text{ g}/\text{m}^{2.7}$ in women and a $LVM > 48 \text{ g}/\text{m}^{2.7}$ in men [17]. RWT was calculated according to the following formula: $RWT = (2 \times LVPWD)/LVDD$. Categories of left ventricular geometry were defined for normal geometry (no LVH, and RWT of <0.42), concentric remodeling (no LVH and RWT >0.42), eccentric hypertrophy (LVH and RWT of <0.42), and concentric hypertrophy (LVH and RWT >0.42) [18]. Fractional shortening (FS) was defined as $([LVDD - LVDS]/LVDD) \times 100$. Progression of LVM, RWT, FS, and IMT was measured as difference between follow-up and baseline values.

2.3. Statistical analysis

Categorical data are given as percentage and continuous data as mean (standard error) or median (p25th, p75th). We used the χ^2 -test for nominal data and the Mann-Whitney-U-test for continuous data for intergroup comparisons by sex. Continuous hormone concentrations were naturally log transformed to normalize their distributions and categorized by age-specific quintiles (for each 10-year age group). First, we implemented age- and multivariable-adjusted linear regression models to examine cross-sectional and longitudinal associations of TT, SHBG, ft, ASD, and DHEAS with five-year change in LVM, RWT, FS, and IMT. Effects were reported as β -coefficients and their 95% confidence interval (CI) for continuous (per log unit increase) and categorized (with quintile three as reference) analyses. We used QQ plots to test the normality of regression residuals. Secondary, we performed Poisson regression models with robust standard errors to assess longitudinal associations of baseline hormone concentrations with incident LVH and plaques. Effects were presented as relative risk (RR) together with 95% CIs per log-unit increase in hormones. The sample was restricted to individuals without prevalent LVH or carotid plaques at baseline, respectively. Thirdly, polytomous logistic regression models were used to assess the association between androgen and SHBG concentrations and altered LV geometry at follow-up (among individuals with normal LV geometry at baseline), using the category “normal geometry” at follow-up as reference group. Presented are odds ratios (OR) and their 95% CIs. Multivariable regression models were adjusted for age, body mass index (BMI), smoking status, diabetes, hypertension, and low-density lipoprotein cholesterol. In longitudinal analyses, multivariable regression models were additionally adjusted for baseline measurements of each outcome variable.

Several separate sensitivity analyses were performed. At this, multivariable regression analyses among women were stratified by hormone therapy ($N = 393$) and oral contraceptive use ($N = 58$). Due to sample attrition from baseline to follow-up, we adjusted the models for possible bias introduced by drop-out, using inverse probability weights. Multiplicative interaction terms between each hormone and covariate were investigated in multivariable models. Given a number of significant interaction terms (p -value < 0.05) between hormones and sex, we performed sex-specific analyses. Additionally, we found significant interaction terms for most hormones and menopausal status in models for LVM and RWT and therefore performed these analyses separately for pre- ($N = 255$) and post-menopausal ($N = 890$) women. Furthermore, all models were additionally adjusted for time of blood sampling.

A p -value < 0.05 was considered statistically significant. This manuscript was written in accordance with the STROBE statement, giving guidelines for reporting of observational studies [19]. All statistical analyses were performed with Stata 13.0 (Stata Corp., College Station, TX, USA).

3. Results

Table 1 presents the characteristics of the full study sample stratified by sex. Overall, we found a higher risk factor burden in men compared to women with regard to waist circumference, blood pressure, and smoking. Androgen concentrations showed an age- and sex-specific distribution (**Table 1**, **Supplementary Figs. 2 and 3**). **Table 2** shows cross-sectional associations between androgens and SHBG and measures of subclinical CVD. At this, we detected an association between ASD and FS in both sexes (**Fig. 1**), as well as between DHEAS and FS after multivariable adjustment (β -coefficient: -1.65 ; 95% CI: -2.78 ; -0.51) (**Supplementary Table 1 and 11**). In multivariable analyses, TT was positively

associated with IMT in women (β -coefficient: 0.02 ; 95% CI: 0.007 ; 0.45) and positively associated with LVM in men (β -coefficient: 1.93 ; 95% CI: 0.07 ; 3.79). As shown in **Table 3**, longitudinal analyses yielded no associations between androgens and SHBG and change in subclinical CVD after multivariable adjustment. However, in categorized analyses, multivariable-adjusted models revealed a positive association between TT quartiles and RWT change (Q2 vs. Q3 (Ref.): β -coefficient: 0.03 ; 95% CI: 0.002 ; 0.06) in women (**Supplementary Table 3**). Altogether, continuous TT concentrations showed no consistent association with subclinical CVD in cross-sectional and longitudinal analyses in both sexes (**Fig. 2**).

From the 624 individuals without carotid plaques at baseline, 345 ($N = 200$ women) developed incident plaques at five-year follow-up. In multivariable models, SHBG was associated with prevalent plaques in women (RR: 0.87 ; 95% CI: 0.79 ; 0.95). An age-adjusted association between SHBG and incident plaques among women was not retained after multivariable adjustment (**Supplementary Table 6**). Similarly, from the 807 individuals without baseline LVH, 216 ($N = 124$ women) developed LVH at five-year follow-up, but multivariable regression models showed no significant associations between androgens, SHBG and incident LVH (**Supplementary Table 7**). Among the 626 individuals ($N = 360$ women) with normal LV geometry at baseline, 261 ($N = 140$ women) developed incident abnormal LV geometry (concentric remodeling, $N = 85$; eccentric hypertrophy, $N = 99$, concentric hypertrophy, $N = 77$). Multivariable polytomous logistic regression models revealed a positive association between baseline SHBG and altered LV geometry at follow-up in men (concentric remodeling: OR: 0.23 ; 95% CI: 0.07 ; 0.77), but not in women (concentric remodeling: OR: 0.95 ; 95% CI: 0.41 ; 2.23) (**Supplementary Fig. 4**). After stratification for menopausal status (**Supplementary Table 8**), as well as after adjustment for hormone therapy, oral contraceptive use, time of blood sampling (**Supplementary Tables 9 and 10**), and inverse probability weighting, the overall estimates including levels of significance remained unchanged (data not shown).

4. Discussion

The present population-based study revealed cross-sectional associations of DHEAS, ft, and ASD with FS as a measure of subclinical CVD. Concentrations of TT were related to baseline IMT only in women. Longitudinal analyses showed a positive association between DHEAS and FS change in men and an inverse association of SHBG with incident carotid plaques in women. These findings support the sex-specific effects of hormones on cardiac and arterial remodeling suggested by previous research [20]. However, despite these findings, we did not detect any consistent associations of androgens with subclinical CVD.

In this study, we found significant interaction terms between sex hormones and SHBG with sex as well as with menopausal status in women. The significant interaction term between sex hormones and sex can be explained through the sex-specific serum hormone concentrations [14,21], sex-specific hormone receptor expression and affinity [22–24], and the different impact of same sex hormones in male or female cells, as well as sex-specific effects of hormones on cardiac and arterial remodeling [20,25]. Additionally, previous studies suggested that extreme and especially low testosterone concentrations in men are associated with an increased CVD risk and higher mortality [26,27], whereas high testosterone concentrations in women are discussed as potential risk factor [28,29]. Regarding the significant interaction term between sex hormones and menopausal status, it is widely known that sex hormone concentrations change during the menopausal transition [30], although it is not clearly understood to date, if menopausal transition increases the risk of CVD independent of

Table 1

Characteristics of the study population, stratified by sex.

Variable	Women (N = 1145)	Men (N = 995)
Age, years	60.7 (9.4)	60.8 (9.3)
Serum total testosterone, nmol/L	0.66 (0.49; 0.96)	14.15 (10.77; 18.27)
Serum SHBG, nmol/L	73.0 (51.06; 101.84)	51.04 (39.42; 67.83)
Serum DHEAS, nmol/L	—	1.23 (0.77; 1.82)
Serum free testosterone, nmol/L	0.007 (0.004; 0.01)	0.21 (0.17; 0.26)
Serum androstenedione, nmol/L	1.23 (0.88; 2.54)	1.67 (1.33; 2.19)
Body mass index, kg/m ²	28.4 (5.1)	28.3 (3.9)
Waist circumference, cm	87.7 (5.1)	98.9 (10.8)
Current smoking, %	15.6	26.5
Oral contraceptive use, %	3.9	—
Hormone replacement therapy, %	36.7	—
Systolic blood pressure, mmHg	139.3 (20.9)	148.5 (20.5)
Diastolic blood pressure, mmHg	83.7 (10.4)	88.6 (11.4)
Antihypertensive medication, %	37.5	34.7
Hypertension, %	50.6	47.4
LDL-C, mmol/L	3.93 (1.1)	3.77 (1.1)
Type 2 diabetes mellitus, %	14.8	17.8
Metabolic syndrome, %	31.6	40.2
Fractional shortening baseline, %	38.3 (7.4)	36.1 (7.8)
Fractional shortening follow-up, %	39.6 (8.5)	37.3 (9.1)
Plaques baseline, %	65.1	73.1
Plaques follow-up, %	81.5	89.1
Relative wall thickness baseline	0.39 (0.1)	0.39 (0.1)
Relative wall thickness follow-up	0.41 (0.1)	0.42 (0.1)
Left ventricular mass baseline, g/m ^{2.7}	46.5 (13.3)	49.5 (13.2)
Left ventricular mass follow-up, g/m ^{2.7}	49.1 (15.7)	51.1 (15.1)
Left ventricular hypertrophy baseline, g/m ^{2.7}	43.7	35.9
Left ventricular hypertrophy follow-up, g/m ^{2.7}	49.2	42.3
Intima media thickness baseline, mm	0.75 (0.15)	0.82 (0.16)
Intima media thickness follow-up, mm	0.77 (0.14)	0.83 (0.17)

Data are percentages, mean (SD) or median (Q1, Q3). Oral contraceptive use was defined according to Anatomical Therapeutic Chemical (ATC) classification code G03AA/B/C/D. Values of these hormones are reported based on availability of each hormone. Serum total testosterone: women N = 829, men N = 790; SHBG: women N = 1028, men N = 932; DHEAS: men N = 915; serum androstenedione: women N = 883, men N = 773; serum free testosterone: women N = 779, men N = 741; free androgen index: women N = 779, men N = 741. SHBG, sex hormone-binding globulin; DHEAS, dehydroepiandrosterone-sulphate.

Table 2

Cross-sectional associations of baseline androgens and SHBG with baseline left ventricular mass, relative wall thickness, fractional shortening, and intima media thickness in men and women.

	Beta coefficient (95% CI)			
	Total testosterone	Androstenedione	SHBG	Free testosterone
Men				
Left ventricular mass				
age-adjusted	-2.79 (-5.95; 0.37)	0.46 (-1.93; 2.86)	-5.06 (-7.46; -2.65)***	0.60 (-2.18; 3.38)
mv-adjusted	1.93 (0.07; 3.79)*	1.51 (-0.63; 3.65)	-1.04 (-3.26; 1.18)	2.94 (0.72; 5.15)**
Relative wall thickness				
age-adjusted	-0.002 (-0.01; 0.01)	0.002 (-0.01; 0.02)	-0.008 (-0.02; 0.006)	0.002 (-0.01; 0.01)
mv-adjusted	0.007 (-0.01; 0.02)	0.002 (-0.01; 0.02)	-0.001 (-0.001; 0.002)	0.008 (-0.01; 0.02)
Fractional shortening				
age-adjusted	0.21 (-1.34; 1.77)	-2.96 (-4.72; -1.20)***	-0.22 (-1.82; 1.37)	0.35 (-1.33; 2.03)
mv-adjusted	-0.66 (-2.25; 0.92)	-2.94 (-4.75; -1.12)***	-0.49 (-2.15; 1.15)	-0.32 (-2.01; 1.36)
Intima media thickness				
age-adjusted	0.001 (-0.01; 0.02)	0.02 (-0.01; 0.04)	-0.003 (-0.02; 0.01)	0.001 (-0.01; 0.01)
mv-adjusted	0.004 (-0.01; 0.02)	0.005 (-0.02; 0.03)	0.007 (-0.01; 0.03)	0.001 (-0.01; 0.02)
Women				
Left ventricular mass				
age-adjusted	-0.02 (-1.86; 1.83)	1.89 (-0.04; 3.82)	-4.16 (-5.58; -2.72)***	2.41 (0.92; 3.91)**
mv-adjusted	-0.34 (-1.93; 1.24)	0.92 (-0.77; 2.59)	0.43 (-0.93; 1.79)	-0.14 (-1.52; 1.23)
Relative wall thickness				
age-adjusted	0.002 (-0.01; 0.01)	0.008 (-0.01; 0.02)	-0.01 (-0.02; -0.005)**	0.009 (-0.002; 0.02)
mv-adjusted	0.001 (-0.01; 0.01)	0.004 (-0.01; 0.02)	-0.008 (-0.02; 0.003)	0.004 (-0.007; 0.01)
Fractional shortening				
age-adjusted	0.93 (-0.12; 1.99)	1.65 (0.57; 2.72)**	-0.58 (-1.46; 0.29)	0.87 (0.02; 1.72)*
mv-adjusted	0.91 (-0.15; 1.99)	1.64 (0.55; 2.73)**	-0.96 (-1.89; 0.16)	0.99 (0.07; 1.91)*
Intima media thickness				
age-adjusted	0.03 (0.01; 0.05)**	0.01 (-0.005; 0.03)	-0.01 (-0.03; 0.004)*	0.02 (0.01; 0.04)***
mv-adjusted	0.02 (0.007; 0.45)**	0.008 (-0.01; 0.03)	-0.003 (-0.02; 0.01)	0.01 (-0.003; 0.02)

Data are β coefficients and their 95% confidence interval with p < 0.05 marked as *, <0.01 **, and <0.001 ***.

The multivariable model was adjusted for age, body mass index, smoking status (three categories), diabetes mellitus, hypertension and low-density lipoprotein cholesterol. Serum total testosterone: women N = 829, men N = 790; SHBG: women N = 1028, men N = 932; DHEAS: men N = 915; serum androstenedione: women N = 883, men N = 773; serum free testosterone: women N = 779, men N = 741. SHBG, sex hormone-binding globulin; CI, confidence interval; mv, multivariable.

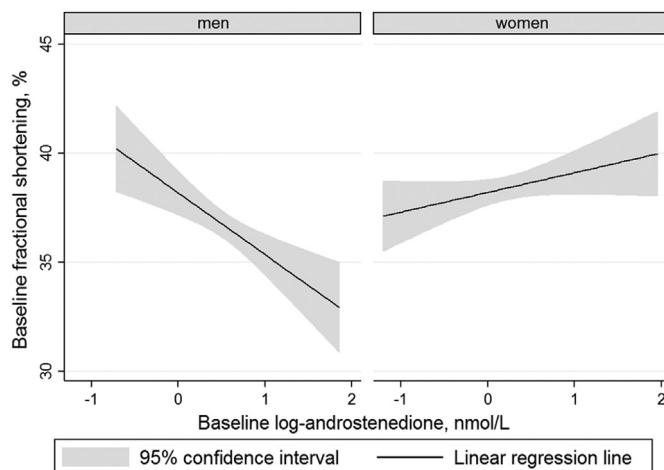


Fig. 1. Continuous relationship of baseline fractional shortening with serum androstenedione. Linear regression for baseline fractional shortening by baseline log-androstenedione concentrations. The black lines represent the continuous relationship and grey areas the 95% confidence interval.

IMT in multivariable-adjusted cross-sectional analyses. These findings are in line with a number of cross-sectional studies in post-menopausal women that suggest an independent positive association between TT and fT with greater IMT [9]. Women with higher androgen concentrations are supposed to have an atherogenic lipid profile including higher insulin and glucose concentrations [31]. Nevertheless, the multivariable-adjusted effects detected in the present study support an association that is independent of BMI, diabetes mellitus, hypertension, and low-density lipoprotein cholesterol. Thus, this is an additional indication that testosterone (T) might have a direct effect on the vasculature via its widespread receptors throughout the cardiovascular system. Previous studies suggested this interaction, showing that T infusion into coronary arteries promotes vasodilatation in men [32]. However, this association is not obligatorily a causative link. Also in line with previous studies, we found no association between androgens and SHBG and IMT in men [7]. In contrast, BMI-dependent associations between SHBG with IMT have been reported in a large observational study among men aged 25–84 years [8]. However, the differences between these results may be due to differences in characteristics of age, BMI, and T assessment.

Table 3

Longitudinal associations of baseline androgens and SHBG with change in left ventricular mass, relative wall thickness, fractional shortening, and intima media thickness in men and women.

	Beta coefficient (95% CI)			
	Total testosterone	Androstenedione	SHBG	Free testosterone
Men				
<i>Left ventricular mass</i>				
age-adjusted	1.56 (-1.06; 4.19)	0.75 (-1.84; 3.36)	2.15 (-0.22; 4.52)	0.89 (-2.30; 4.09)
mv-adjusted	1.05 (-1.40; 3.52)	1.00 (-1.63; 3.64)	1.58 (-0.96; 4.12)	0.80 (-1.87; 3.49)
<i>Relative wall thickness</i>				
age-adjusted	-0.008 (-0.03; 0.01)	0.01 (-0.01; 0.04)	-0.006 (-0.03; 0.01)	-0.004 (-0.03; 0.02)
mv-adjusted	-0.006 (-0.02; 0.01)	0.01 (-0.004; 0.04)	-0.01 (-0.03; 0.006)	0.006 (-0.01; 0.03)
<i>Fractional shortening</i>				
age-adjusted	0.24 (-2.11; 2.60)	1.92 (-1.03; 4.89)	0.94 (-1.33; 3.23)	-0.58 (-3.16; 1.99)
mv-adjusted	-0.23 (-2.58; 2.10)	-0.07 (-2.86; 2.72)	0.73 (-1.43; 2.91)	-0.62 (-2.98; 1.73)
<i>Intima media thickness</i>				
age-adjusted	0.01 (-0.009; 0.03)	0.002 (-0.02; 0.02)	-0.01 (-0.009; 0.03)	0.01 (-0.005; 0.04)
mv-adjusted	0.01 (0.009; 0.03)	0.001 (-0.02; 0.02)	0.01 (-0.01; 0.03)	0.02 (-0.003; 0.04)
Women				
<i>Left ventricular mass</i>				
age-adjusted	0.32 (-1.91; 2.55)	0.34 (-1.80; 2.50)	-1.01 (-2.75; 0.72)	0.38 (-1.37; 2.14)
mv-adjusted	0.16 (-1.86; 2.20)	0.35 (-1.78; 2.49)	-0.68 (-2.48; 1.12)	0.22 (-1.48; 1.92)
<i>Relative wall thickness</i>				
age-adjusted	-0.003 (-0.02; 0.01)	-0.01 (-0.04; 0.007)	0.01 (-0.005; 0.03)	-0.01 (-0.03; 0.003)
mv-adjusted	0.003 (-0.01; 0.02)	-0.004 (-0.02; 0.01)	-0.003 (-0.02; 0.01)	0.001 (-0.01; 0.01)
<i>Fractional shortening</i>				
age-adjusted	0.53 (-1.11; 2.69)	-1.01 (-2.79; 0.77)	0.74 (-0.79; 2.27)	0.04 (-1.24; 1.32)
mv-adjusted	1.22 (-0.25; 2.69)	-0.01 (-1.52; 1.51)	0.12 (-1.50; 1.74)	0.57 (-0.71; 1.86)
<i>Intima media thickness</i>				
age-adjusted	-0.003 (-0.02; 0.01)	0.004 (-0.01; 0.01)	0.001 (-0.01; 0.01)	-0.0001 (-0.01; 0.01)
mv-adjusted	0.004 (-0.01; 0.02)	0.009 (-0.004; 0.02)	-0.008 (-0.02; 0.006)	0.007 (-0.009; 0.02)

Data are β coefficients and their 95% confidence interval with $p < 0.05$ marked as *.

The multivariable model was adjusted for age, body mass index, smoking status (three categories), diabetes mellitus, hypertension, low-density lipoprotein cholesterol and the baseline outcome variable, respectively. Serum total testosterone: women N = 829, men N = 790; SHBG: women N = 1028, men N = 932; DHEAS: men N = 915; serum androstenedione: women N = 883, men N = 773; serum free testosterone: women N = 779, men N = 741.

DHEAS, dehydroepiandrosterone-sulphate; SHBG, sex hormone-binding globulin; CI, confidence interval; mv, multivariable.

normal aging or central adiposity. At this, analyses stratified for sex and menopausal status, respectively, revealed sex-specific results but no differences in levels of significance in pre-menopausal compared to post-menopausal women.

4.1. Cross-sectional analyses

4.1.1. Associations of androgens and SHBG with IMT

In women, the present study showed an association of TT with

4.1.2. Associations of androgens and SHBG with carotid plaques

Furthermore, the present study revealed a cross-sectional association between SHBG and prevalent plaques in women. Previous studies showed that plaque characteristics are sex-specific, suggesting that women in general show less plaque than men [25]. In addition to observational findings, a previous study on SHBG gene polymorphisms observed an association of the SHBG gene promoter polymorphism with markers of atherosclerosis in post-menopausal women [33]. Overall, the present findings add to an increasing body

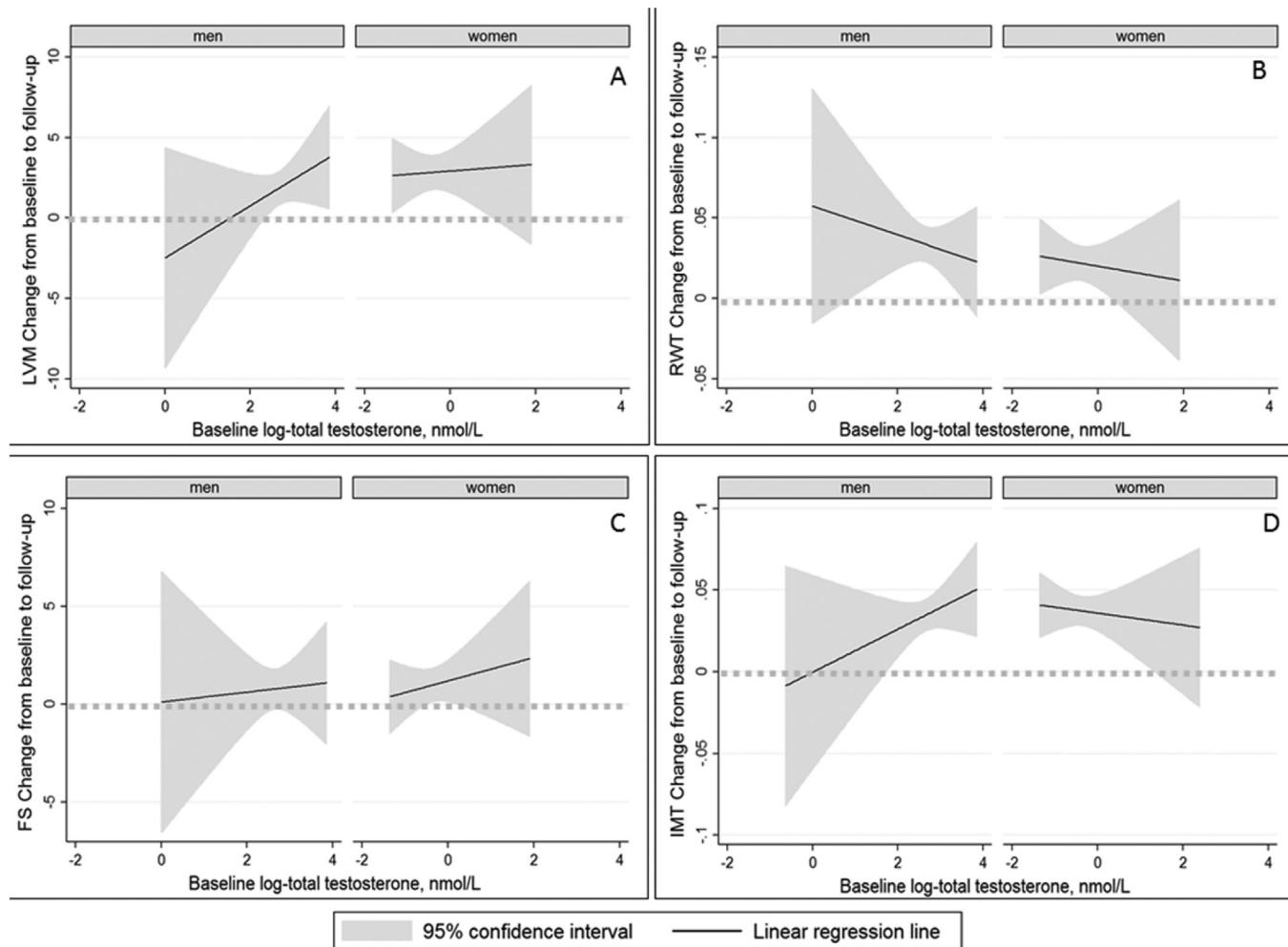


Fig. 2. Scatterplot for five-year change in left ventricular mass, relative wall thickness, fractional shortening and intima media thickness by total testosterone concentrations. Continuous relationship for absolute difference in (A) left ventricular mass (LVM), (B) relative wall thickness (RWT), (C) fractional shortening (FS) and (D) intima media thickness (IMT) by baseline log-total testosterone (TT) concentrations, separately in men and women. Confidence intervals crossing the grey zero-lines illustrate non-significance.

of evidence suggesting SHBG more strongly related to subclinical CVD including carotid plaques than androgens itself. Since SHBG's high affinity binding of sex hormones does not keep them absolutely inactive, a still unknown biochemical potential to enter for example endocytic pathways [34] or at least regulate levels of free sex hormones is still given [35]. Consequently, androgens and SHBG are highly correlated. But despite the suggested independent effect of SHBG, partly explained by its receptors [36], further research is indicated to precise its role in the pathogenesis of CVD.

4.1.3. Associations of androgens and SHBG with FS

Furthermore, we observed an inverse association of DHEAS with FS in men. This finding supports previous studies indicating that DHEAS may play a physiological role in the prevention of heart disease in men [37], but not in women [38]. Additionally, the present sex-specific associations between ASD and FS, observed in cross-sectional analyses, add to the existing literature on the sex-specific effects of sex hormones on cardiac and arterial remodeling [20], whose physiological mechanisms are not clearly understood to date.

4.2. Longitudinal analyses

In longitudinal analyses we did not observe any consistent

associations of androgens and SHBG with subclinical CVD. Only age-adjusted models showed an association of SHBG with incident plaque and DHEAS with FS change, respectively. However, both associations were not retained after multivariable adjustment in cross-sectional analyses. Additionally, both associations appeared to be not independent of BMI, suggesting that over the five-year follow-up, especially visceral obesity might influence the association between SHBG, DHEAS and subclinical CVD, since particularly SHBG [39] and DHEA are correlated with obesity [40].

In line with previous research [41] we found no association between androgens or SHBG and IMT. Previous longitudinal studies suggested an inverse association of SHBG and IMT in settings with pre- or peri-menopausal women [10,42], whereas we did not observe such an association. These divergent findings may be in part explained by differences in study characteristics (especially age structure) and sex hormone measurement. Androgen and SHBG concentrations, and most prominently T [43], changes with advanced age and increased comorbidity [44]. However, in the present study we could not gather changes in androgen concentrations over the five-year follow-up, since androgens and SHBG were only measured at baseline. The divergent findings for the association between androgens and subclinical CVD in men and women in cross-sectional and longitudinal analyses might be explained by the healthy responder effect and unmeasured

confounding during follow-up. Both factors apply to a population-based cohort study comprising comparatively healthy participants from the general population like the present sample. Additionally, unmeasured confounders, as well as change in sex hormone status, may dilute potential longitudinal associations between androgens and subclinical CVD, as observed during cross-sectional analyses.

4.3. Strengths & limitations

The strengths of the present study include the population-based approach, the longitudinal study design and the accurate assessment of subclinical outcome variables, performed by trained and certified examiners. Additionally, TT and ASD were measured via LC-MS instead of immunoassay, which allows an accurate assessment of androgen status, especially in women [11]. Limitations might have arisen from single determination of sex hormones and SHBG, as they were measured only at baseline. Additionally, lipid concentrations may vary because of varying fasting times. The recruitment procedure in SHIP-0 did not allow to collect fasting blood samples. Blood samples were taken between 07:00 a.m. and 04:00 p.m. However, based on previous studies reporting only minor differences between fasting and non-fasting participants [45,46], we expected that the fasting status is unlikely to cause independent associations in our study. Sex hormone concentrations may vary because of varying time of blood sampling. However, additional adjustment "time of blood sampling" showed no significant impact on the estimates. Additionally, a previous investigation in SHIP showed only minor differences in TT levels between serum samples drawn before midday and afternoon [47], therefore, this variation is expected to be minimal. Furthermore, only 23% of the final study sample comprised pre-menopausal women. However, women in the critical time window for CVD events (peri- and post-menopausal women) were completely involved in the analyses and are representative for the age group ≥ 45 years. As SHIP-0 did not collect any information about polycystic ovary syndrome (PCOS) criteria, we could not include potential effects of PCOS on SHBG in the present analyses. SHBG concentrations are typically lowered in women with PCOS due to the effects of obesity and subsequently increased insulin responses to a decreased hepatic production of SHBG [48]. Despite the lack of PCOS data, we carefully adjusted for waist-circumference, involving the link between SHBG concentrations and obesity in all analyses.

5. Conclusions

Based on high-quality LC-MS measurements and valid subclinical CVD measures, the present observational study among men and women from the general population found cross-sectional associations of TT with IMT and SHBG with carotid plaques in women and of ADS with FS in both sexes.

In longitudinal analyses, no consistent associations of endogenous androgen and SHBG concentrations with subclinical measures of CVD were observed. These observational results support recent trial data suggesting testosterone administration in men being unrelated to subclinical CVD outcomes [49]. To further elucidate the potential role and the clinical and epidemiological significance of androgens and SHBG in the pathogenesis of CVD, future epidemiological meta-analysis, as well as clinical studies of hormone therapy among well-defined patient populations might reveal much needed insights.

Disclosure statement

The authors have nothing to disclose.

Acknowledgements

SHIP is part of the Community Medicine Research net (CMR) of the University of Greifswald, Germany, which is funded by the Federal Ministry of Education and Research (grants no. 01ZZ9603, 01ZZ0103, and 01ZZ0403), the Ministry of Cultural Affairs, as well as the Social Ministry of the Federal State of Mecklenburg-West Pomerania. This work is also part of the research project Greifswald Approach to Individualized Medicine (GANI_MED), and was supported by the DFG (German Research Foundation), the DZHK (German Centre for Cardiovascular Research), and by the BMBF (German Ministry of Education and Research).

Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.atherosclerosis.2016.02.020>.

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Association of sex hormones with incident 10-year cardiovascular disease and mortality in women



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ARTICLE INFO

Article history:

Received 13 May 2015

Received in revised form 20 August 2015

Accepted 21 August 2015

Keywords:

Testosterone

Sex hormones

Women

Cardiovascular disease

Epidemiology

ABSTRACT

Objectives: The aims of this study were to ascertain whether women with high levels of serum total testosterone (TT) or low levels of sex hormone-binding globulin (SHBG) are more likely to develop cardiovascular disease (CVD), and to investigate potential associations between sex hormones and mortality (all-cause, as well as cause-specific) in the general population.

Study design and main outcome measures: Data on 2129 women with a mean age of 49.0 years were obtained from the population-based Study of Health in Pomerania over a median follow-up of 10.9 years. Associations of baseline levels of TT, SHBG, and rostenedione (ASD), and free testosterone (fT), and of the free androgen index (FAI), with follow-up CVD morbidity, as well as all-cause and CVD mortality, were analyzed using multivariable regression modeling.

Results: At baseline the prevalence rate of CVD was 17.8% (378 women) and the incidence of CVD over the follow-up was 50.9 per 1000 person-years. We detected an inverse association between SHBG and baseline CVD in age-adjusted models (relative risk per standard deviation increase: 0.83; 95% confidence interval: 0.74–0.93). We did not detect any significant associations between sex hormone concentrations and incident CVD in age- and multivariable-adjusted Poisson regression models. Furthermore, none of the sex hormones (TT, SHBG, ASD, fT, FAI) were associated with all-cause mortality.

Conclusions: This population-based cohort study did not yield any consistent associations between sex hormones in women and incident CVD or mortality risk.

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1. Introduction

Studies of testosterone in men have shown that both low and high levels are associated with a greater burden of risk factors for CVD and indeed an increased CVD mortality rate [1]. Furthermore, testosterone has been declared a potential biomarker for the development of CVD risk factors [2]. Our interest was to ascertain whether endogenous sex hormones and sex hormone-binding globulin (SHBG) lead to similar observations regarding CVD in

women. Previous observational studies have shown an association between sex hormone concentrations and subclinical as well as clinical CVD. That is, women with high serum total testosterone (TT) and low SHBG concentrations have been reported to have a higher burden of CVD risk factors and a higher incidence of CVD itself [3–7]. However, it is not clear whether these observational findings translate into an increased all-cause mortality risk associated with sex hormone concentrations in women. There have been conflicting results. For instance, while one study among elderly diabetic women suggested that low serum levels of free testosterone (fT) were a marker for increased mortality risk [8], others observed no association between sex hormones and all-cause mortality in women [9].

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The limitations of these previous studies have included the measurement of the sex hormones by radioimmunoassay (RIA), the use of highly selective community- or patient-based samples, small sample sizes, and cross-sectional study design. The potential association between sex hormones and incident CVD and mortality risk in women in the general population is therefore yet to be properly explored. To overcome these limitations, we investigated the potential association between sex hormone concentrations in women and incident CVD and mortality risk using a large population-based sample; furthermore, sex hormone concentrations were measured by LC-MS/MS. The study drew on 10-year follow-up data from the longitudinal Study of Health in Pomerania (SHIP). Of note, this is the first outcome study on the role of testosterone as a potential cardiovascular risk marker in women to substantially build on recently established age-specific reference ranges [10].

2. Methods

2.1. Study population

The Study of Health in Pomerania (SHIP) is a population-based cohort study in north-eastern Germany. We have previously published details of the study design, recruitment, and procedures [11]. In brief, from the target population of individuals with German citizenship and main residency in the study area of West Pomerania, comprising 213,057 inhabitants in 1996, a two-stage stratified cluster sample was drawn of adults aged 20–79 years. The net sample comprised 6265 eligible individuals (3160 women). Of these, 4308 individuals (2192 women) participated between 1997 and

2001 in the baseline study (response rate 68.8%), after written informed consent was obtained from each participant. The Ethics Committee of the University of Greifswald authorized the study protocol, which is consistent with the principles of the Declaration of Helsinki. Women with the following characteristics were excluded (with some overlap between groups): pregnancy ($N=18$), self-reported bilateral oophorectomy or hysterectomy ($N=52$), and all women who had received the following prescribed drugs in the last seven days, based on the medication packages or, if not available, on self-statement and categorized based on the Anatomical Therapeutic Chemical [6] classification index—sex hormone antagonists ($N=8$), glucocorticoids ($N=23$), and natural opium alkaloids ($N=14$). The final study population consisted of 2129 female baseline participants, of whom 1711 were re-examined (a 76.6% response rate) at five-year follow-up (between 2002 and 2006) and 1235 (54.2% response rate) at 10-year follow-up (between 2008 and 2012).

2.2. Measures

A computer-assisted personal interview was conducted to assess socio-demographic and behavioral characteristics and medical history (e.g., information about sex, age, alcohol consumption, physical activity, pregnancy, gynecological surgery, bilateral oophorectomy and/or hysterectomy, medication use). Women were divided into categories of current smokers of cigarettes, former smokers, and never smokers. Mean daily alcohol consumption was calculated using beverage-specific pure ethanol volume proportions [12]. Women were classified as physically active if they participated in physical training for at least one hour a week

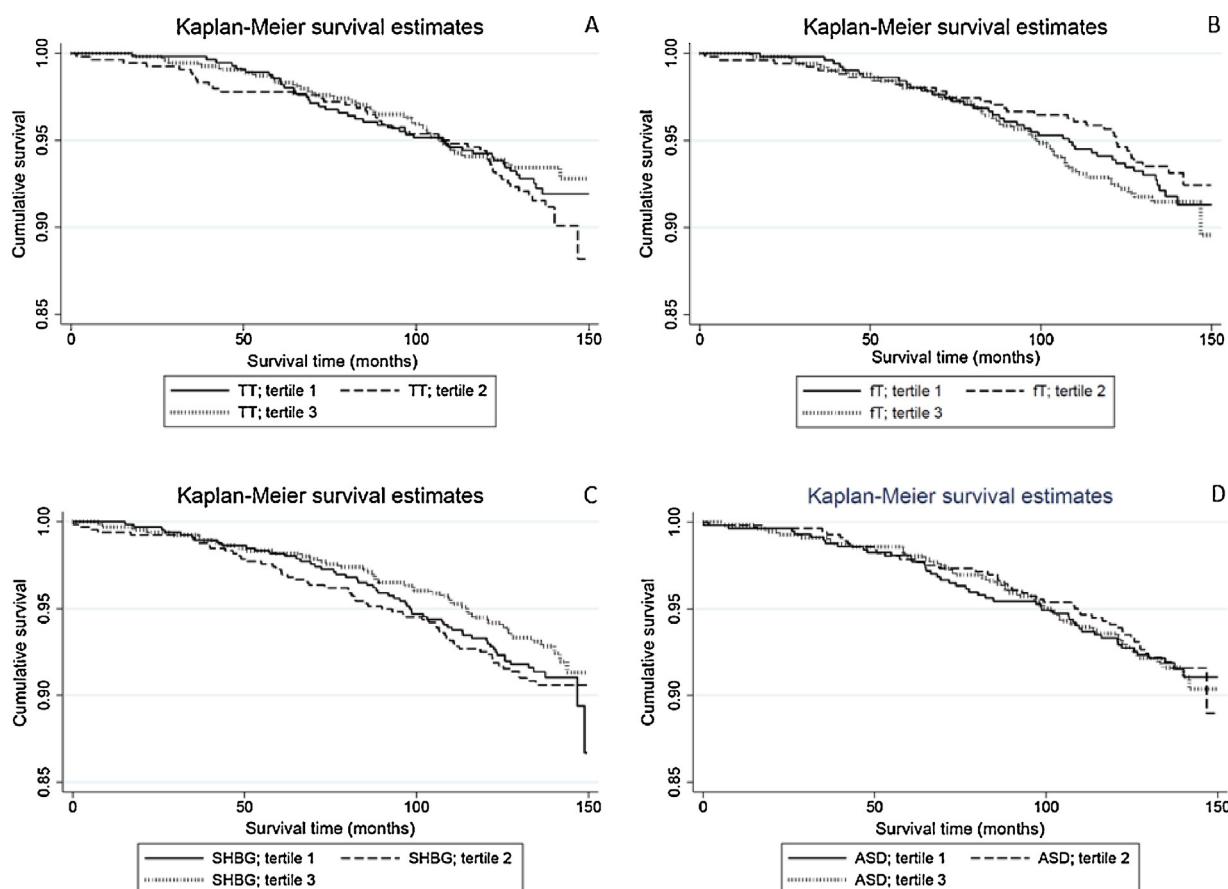


Fig. 1. Kaplan-Meier survival curves for sex hormone tertiles. (A) Total testosterone (TT) tertiles, (B) sex hormone-binding globulin (SHBG) tertiles, (C) free testosterone (FT) tertiles, (D) androstenedione (ASD) tertiles. Survival times did not differ significantly between the groups.

Table 1
Baseline characteristics of the study population.

Variable	All women (N=2129)	Pre-menopausal women (N=1120)	Post-menopausal women (N=1006)	Women without prevalent CVD (N=1727)	Women with prevalent CVD (N=378)
Age, years	49.0 (16.1)	36.5 (9.5)	62.5 (9.4)	46.5 (15.5)	58.8 (14.7)
Body mass index, kg/m ²	26.1 (5.4)	25.3 (5.0)	28.7 (5.1)	26.3 (5.1)	29.4 (5.8)
Waist circumference, cm	81.5 (13.1)	78.3 (12.0)	88.5 (12.2)	81.6 (12.4)	90.0 (14.1)
Serum total testosterone, nmol/L	0.76 (0.55; 1.06)	0.82 (0.6; 1.13)	0.68 (0.5; 0.96)	0.77 (0.56; 1.05)	0.69 (0.5; 1.05)
Serum SHBG, nmol/L	83.2 (58.0; 123.5)	100.4 (67.3; 153.6)	71.0 (50.3; 97.9)	86.9 (60.3; 129.0)	70.2 (48.1; 100.4)
Serum free testosterone, nmol/L	0.007 (0.004, 0.011)	0.006 (0.004, 0.01)	0.007 (0.004, 0.011)	0.006 (0.004, 0.01)	0.007 (0.005, 0.01)
Serum androstenedione, pmol/L	1.61 (1.05; 2.50)	1.19 (0.87; 1.65)	2.24 (1.49; 3.17)	1.7 (1.12; 2.63)	1.36 (0.9; 1.94)
Current smoking, %	27.3	37.0	16.3	29.7	16.4
Alcohol consumption, g/day	2.24 (0.0; 7.5)	2.48 (0.0; 8.7)	0.0 (0.0; 4.9)	2.48 (0; 7.45)	0 (0; 4.9)
Physically active, %	22.8	27.3	17.8	24.1	17.2
Oral contraceptive use, %	17.3	42.9	1.3	38.5	28.0
Hormone therapy, %	11.2	7.91	16.34	12.9	11.0
Diastolic blood pressure, mmHg	80.7 (10.7)	79 (10.0)	84 (10.8)	81.0 (10.4)	82.8 (11.5)
Systolic blood pressure, mmHg	127.3 (21.4)	121.2 (15.9)	141.6 (21.6)	128.9 (20.4)	138.7 (22.4)
Antihypertensive medication, %	19.9	9.9	39.8	18.7	46.8
Hypertension, %	38.6	25.5	53.1	33.6	59.4
Diabetes, %	9.83	1.34	18.34	6.43	22.22
Cardiovascular disease, %	17.8	7.9	29.1		
Angina pectoris	4.6	2.59	6.76		
Peripheral artery disease	1.8	0.36	3.38		
Heart failure	13.5	5.6	22.3		
Stroke	1.7	0.36	2.9		
Myocardial infarction	1.4	0	2.9		

Data are percentages, mean (SD), or median (Q1, Q3).

Oral contraceptive use was defined according to Anatomical Therapeutic Chemical [6] classification code G03AA/B/C/D. Hormone therapy was defined according to Anatomical Therapeutic Chemical [6] classification code G03C/D/F. Values of these hormones are reported based on availability of each hormone. Serum total testosterone (TT), N=1638; SHBG, N=1970; serum androstenedione (ASD), N=1695; serum free testosterone (FT), N=1525; free androgen index (FAI), N=1525. CVD, Cardiovascular disease; SHBG, sex hormone-binding globulin.

during summer or winter. To stratify the sample into pre- and post-menopausal women, we applied a previously published categorization: all women <40 years of age and between 40 and 60 years who reported having a menstrual cycle were classified as pre-menopausal; and all women ≥60 years of age, together with all women between 40 and 60 years who reported having no menstrual cycle, were classified as post-menopausal [13]. We assessed the use of oral contraceptive (G03A) and hormone therapy (G03C, G03D, or G03F) based on ATC codes [10]. Weight was measured utilizing standard digital scales (to the nearest 0.1 kg); women were weighed in light clothing and without shoes. Waist circumference was measured utilizing a tape midway between the lower rib margin and the iliac crest in the horizontal plane and height was measured using a digital ultrasound instrument. Body mass index (BMI) was calculated from the body weight in kilograms and height in meters [BMI = kg/m²]. After a resting period of at least five

minutes, systolic and diastolic blood pressure were measured three times (at an interval of 3 min) on the right arm of seated subjects by use of an oscillometric digital blood pressure monitor (HEM-705CP, Omron Corporation, Tokyo, Japan). For the present analyses the mean of the second and third measurements was used. Blood pressure ≥140/90 mmHg or use of antihypertensive medication (ATC codes C02–C09) was defined as hypertension [14,15]. CVD was assessed by self-reported symptoms of angina pectoris, peripheral artery disease, or heart failure, and self-reported physician diagnosis of stroke and myocardial infarction:

- Angina pectoris: self-reported chest pain (middle/left chest, radiating pain into the left arm).
- Peripheral artery disease: self-reported leg pain while walking (intermittent claudication), self-reported leg pain that made the

Table 2

Associations of sex hormone concentrations in women with baseline CVD.

	Total testosterone	Sex hormone-binding globulin Relative risk (95% confidence interval)	Androstenedione	Free testosterone	Free androgen index
	Age-adjusted model				
Contin.	1.03 (0.93; 1.13)	0.83 (0.74; 0.93)*	0.98 (0.87; 1.11)	1.16 (1.04; 1.30)*	1.19 (1.06; 1.34)*
Tertile 1	1.22 (0.95; 1.56)	1.36 (1.09; 1.69)*	1.20 (0.94; 1.53)	0.74 (0.57; 0.96)*	0.84 (0.64; 1.10)
Tertile 2	Ref.	Ref.	Ref.	Ref.	Ref.
Tertile 3	1.15 (0.89; 1.49)	0.93 (0.73; 1.19)	1.21 (0.94; 1.55)	0.88 (0.68; 1.13)	1.09 (0.84; 1.40)
P for trend	0.61	0.97	<0.01	0.22	0.06
Multivariable-adjusted model					
Contin.	1.01 (0.91; 1.12)	0.94 (0.85; 1.05)	0.98 (0.86; 1.10)	1.04 (0.92; 1.17)	1.04 (0.93; 1.18)
Tertile 1	1.16 (0.91; 1.49)	1.15 (0.92; 1.43)	1.22 (0.95; 1.55)	0.84 (0.65; 1.09)	1.01 (0.77; 1.32)
Tertile 2	Ref.	Ref.	Ref.	Ref.	Ref.
Tertile 3	1.05 (0.81; 1.35)	1.03 (0.80; 1.32)	1.22 (0.95; 1.57)	0.79 (0.61; 1.01)	0.98 (0.77; 1.26)
P for trend	0.37	0.97	0.32	0.56	0.83

The multivariable model is adjusted for age, smoking status, waist circumference, physical activity and, alcohol consumption.

The presented data show the relative risk (95% confidence interval) with $p < 0.05$ marked as *.

Ref., reference; contin., continuous.

Table 3

Associations of baseline sex hormone concentrations with follow-up CVD.

	Total testosterone	Sex hormone-binding globulin Relative risk (95% confidence interval)	Androstenedione	Free testosterone	Free androgen index
Age-adjusted model					
Contin.	1.00 (0.96; 1.05)	1.01 (0.97; 1.06)	0.99 (0.93; 1.05)	0.99 (0.95; 1.05)	0.99 (0.95; 1.05)
Tertile 1	1.16 (0.69; 1.94)	1.22 (0.79; 1.87)	1.25 (0.77; 2.03)	0.97 (0.57; 1.66)	0.99 (0.57; 1.71)
Tertile 2	Ref.	Ref.	Ref.	Ref.	Ref.
Tertile 3	1.00 (0.57; 1.76)	0.68 (0.42; 1.11)	0.99 (0.57; 1.70)	1.24 (0.72; 2.12)	1.44 (0.84; 2.45)
P for trend	0.55	0.01	0.34	0.36	0.15
Multivariable-adjusted model					
Contin.	1.00 (0.96; 1.06)	1.02 (0.97; 1.07)	1.07 (0.93; 1.09)	0.99 (0.95; 1.05)	0.99 (0.94; 1.05)
Tertile 1	1.11 (0.67; 1.85)	1.08 (0.69; 1.69)	1.07 (0.65; 1.78)	0.86 (0.47; 1.49)	0.89 (0.50; 1.59)
Tertile 2	Ref.	Ref.	Ref.	Ref.	Ref.
Tertile 3	0.92 (0.51; 1.63)	0.63 (0.37; 1.07)	0.95 (0.55; 1.64)	0.97 (0.55; 1.08)	1.15 (0.66; 2.01)
P for trend	0.44	0.06	0.64	0.66	0.35

The multivariable model is adjusted for age, smoking status, waist circumference, physical activity and, alcohol consumption.

The presented data show the relative risk (95% confidence interval) with $p < 0.05$ marked as *.

Ref., reference; contin., continuous.

subject slow down or stop walking, self-reported walking distance without pain <200 m.

- Heart failure: self-reported dyspnea with shortness of breath at a medium or low level of physical activity.
- Stroke: self-reported physician diagnosis of stroke.
- Myocardial infarction: self-reported physician diagnosis of myocardial infarction.

This definition of CVD has been used in several previous studies [16,17].

A detailed description of the TT and androstenedione (ASD) measurements has been previously published [10]. Briefly, serum aliquots (stored at -80°C) were prepared from a blood sample taken from the cubital vein with the woman in a supine position between 8:00 a.m. and 7:00 p.m. Measurements of serum TT and ASD concentrations were carried out between December 2010 and March 2011 in the Department of Clinical Chemistry at the University Hospital of South Manchester (Manchester, UK). Liquid chromatography–tandem mass spectrometry (LC–MS/MS) using a validated routine method was performed [18], with the result that the standard curve was linear to 50.0 nmol/L, the lower limit of quantification was 0.25 nmol/L, and intra- and inter-assay coefficients of variation were <10% for both TT and ASD over the range 0.3–35 nmol/L. SHBG concentrations were measured from frozen serum aliquots using a radioimmuno assay on an Advia Centaur (Siemens, Eschborn, Germany) with an inter-assay coefficient of variation of 6.6% at the 27.1 nmol/L level, 7.6% at the

48.2 nmol/L level, and 7.7% at the 52.3 nmol/L level. FT and free androgen index (FAI) were calculated from measured TT and SHBG concentrations: [free T (nmol/L) = $((-a + \sqrt{b})/c)/10^{-9}$ with $a = \text{SHBG}$ (nmol/L) – TT (nmol/L) + 23.43, $b = a^2 + (4 \times 23.43 \times \text{TT} (\text{nmol/L}))$ and $c = 2 \times 23.43 \times 10^9$ for a standard average albumin concentration of 4.3 g/dL [19]; [FAI = TT × 100/SHBG] [19].

Individuals were included in the analysis until the time of death or up to study end or last follow-up. Death certificates collected from the local health authority (according to place of residence at time of death) were coded according to the International Classification of Diseases (ICD-10) by a certified nosologist. Thereafter, two internists (H.W. & M.D.) independently validated the underlying cause of death, and performed a joint reading in cases of disagreement. A third internist (H.V.) ruled on cause in cases where agreement could not be reached.

2.3. Statistical analysis

Categorical data were recorded as percentages and continuous data as means (standard deviation) or medians (p25th, p75th). Cox proportional regression models were used to assess the association between sex hormone concentrations and all-cause mortality over the median follow-up period of 10.9 years. We graphically presented Kaplan–Meier analyses and compared the survival curves using the log-rank test. Continuous sex hormone concentrations were categorized as age-specific tertiles (for each 10-year age group) with tertile 2 as the reference group. We

Table 4

Associations of sex hormone concentrations with all-cause mortality.

	Total testosterone	Sex hormone-binding globulin Hazard ratio (95% confidence interval)	Androstenedione	Free testosterone	Free androgen index
Age-adjusted model					
Contin.	1.05 (0.89; 1.24)	0.92 (0.76; 1.11)	1.08 (0.88; 1.33)	1.07 (0.88; 1.34)	1.08 (0.88; 1.34)
Tertile 1	0.81 (0.53; 1.23)	0.88 (0.62; 1.27)	1.02 (0.68; 1.53)	1.21 (0.76; 1.92)	1.19 (0.75; 1.87)
Tertile 2	Ref.	Ref.	Ref.	Ref.	Ref.
Tertile 3	0.74 (0.48; 1.14)	0.73 (0.50; 1.07)	1.12 (0.74; 1.69)	1.36 (0.89; 2.13)	1.31 (0.84; 2.05)
P for trend	0.72	0.33	0.65	0.59	0.65
Multivariable-adjusted model					
Contin.	1.08 (0.90; 1.29)	1.03 (0.83; 1.27)	1.09 (0.88; 1.35)	1.04 (0.83; 1.30)	1.02 (0.82; 1.29)
Tertile 1	0.79 (0.51; 1.22)	0.87 (0.02; 1.27)	1.00 (0.65; 1.54)	1.37 (0.85; 2.22)	1.34 (0.83; 2.16)
Tertile 2	Ref.	Ref.	Ref.	Ref.	Ref.
Tertile 3	0.78 (0.50; 1.22)	0.92 (0.61; 1.58)	1.22 (0.80; 1.86)	1.37 (0.86; 2.19)	1.30 (0.82; 2.08)
P for trend	0.97	0.77	0.36	0.96	0.96

The multivariable model is adjusted for age, smoking status, waist circumference, physical activity and, alcohol consumption.

The presented data show the hazard ratio (95% confidence interval) with $p < 0.05$ marked as *.

Ref., reference; contin., continuous.

Table 5

Associations of sex hormone concentrations in women with CVD-mortality.

	Total testosterone	Sex hormone-binding globulin Hazard ratio (95% confidence interval)	Androstanedione	Free testosterone	Free androgen index
Age-adjusted model					
Contin.	1.07 (0.81; 1.40)	0.81 (0.59; 1.12)	1.08 (0.77; 1.52)	1.35 (0.96; 1.91)	1.40 (0.98; 1.99)
Tertile 1	0.80 (0.41; 1.58)	1.03 (0.58; 1.82)	0.96 (0.49; 1.86)	1.10 (0.52; 2.32)	1.15 (0.54; 2.46)
Tertile 2	Ref.	Ref.	Ref.	Ref.	Ref.
Tertile 3	0.81 (0.41; 1.62)	0.69 (0.36; 1.32)	1.30 (0.68; 2.48)	1.62 (0.81; 3.24)	1.86 (0.92; 3.74)
P for trend	0.96	0.23	0.37	0.26	0.16
Multivariable-adjusted model					
Contin.	1.16 (0.87; 1.33)	0.96 (0.67; 1.38)	1.12 (0.79; 1.57)	1.33 (0.93; 1.92)	1.36 (0.93; 1.98)
Tertile 1	0.66 (0.32; 1.33)	0.98 (0.54; 1.75)	0.85 (0.42; 1.70)	1.07 (0.50; 2.29)	1.19 (0.54; 2.63)
Tertile 2	Ref.	Ref.	Ref.	Ref.	Ref.
Tertile 3	0.80 (0.40; 1.61)	0.91 (0.48; 1.92)	1.39 (0.72; 2.69)	1.52 (0.75; 3.07)	1.76 (0.87; 3.58)
P for trend	0.59	0.97	0.16	0.31	0.24

The multivariable model is adjusted for age, smoking status, waist circumference, physical activity and, alcohol consumption.

The presented data show the hazard ratio (95% confidence interval) with $p < 0.05$ marked as *.

Ref., reference; contin., continuous.

used tertile 2 as the reference category because different sex hormones showed different directions of risk with regard to the same outcome of interest. We analyzed all regression models after log-transforming the highly skewed sex hormone concentrations. In addition, hazard ratios (HRs) are reported for standard deviation (SD) increases in sex hormone levels, with their 95% confidence intervals (CIs). To satisfy the proportional hazards assumption, log-log plots were graphically explored. We confirmed the proportional hazard assumption with Schoenfeld's tests and visual inspection of smoothed estimates of the HR against time. Multivariable regression models were adjusted for age, waist circumference, smoking, physical activity, and alcohol consumption and in a second model additionally for cholesterol and systolic blood pressure. All models were rerun for cause-specific mortality analyses. We calculated p -values for trends by incorporating sex hormone tertiles, as an ordinal score, in the regression models. To test for cross-sectional and longitudinal associations of sex hormone levels with CVD as dependent variables, we implemented age- and multivariable-adjusted generalized Poisson regression models with robust standard errors and reported estimates as relative risks (RRs) and their 95% CIs. For analyses of the incidence of CVD the sample was restricted to women without CVD at baseline.

Several separate sensitivity analyses were performed. Multivariable regression models were repeated and stratified by menopausal status, use of hormone therapy ($N=618$), and self-reported use of oral contraceptives. $P < 0.05$ was considered statistically significant. All the statistical analyses were performed with Stata 13.0 (Stata Corp., College Station, TX, USA).

3. Results

Table 1 presents the baseline characteristics of the full study sample. 47.3% (1006 women) were premenopausal and the other 52.7% (1120 women) postmenopausal. The prevalence of CVD at baseline was 17.8% ($N=378$), and its incidence was 50.9 per 1000 person-years over the median follow-up period of 10.9 years ($p25: 10.6$, $p75: 11.5$). The total number of incident CVD events was 94 at 10-year follow-up. **Fig. 1** presents Kaplan–Meier survival curves for all-cause mortality by sex hormone tertiles. In age-adjusted models, we detected a negative association between SHBG (RR: 0.83; 95% CI: 0.74–0.93) and prevalent CVD, and a positive association between fT (RR: 1.16; 95% CI: 1.04–1.13) and FAI (RR: 1.19; 95% CI: 1.06–1.34) and prevalent CVD. However, these associations were not retained after multivariable adjustment (**Table 2**). The confounder most responsible for the attenuation in significance level was waist circumference. As shown in **Table 3**, no significant associations

were detected between sex hormone concentrations and the 10-year risk of incident CVD. The numbers of cases ascertained by the self-reported symptoms used for the definition of CVD are shown in Supplementary Table 3.

Table 4 shows the associations between sex hormone concentrations and all-cause mortality. We observed no significant associations in age-adjusted models between mortality and TT (HR: 1.05; 95% CI: 0.89–1.24), SHBG (HR: 0.92; 95% CI: 0.76–1.11), fT (HR: 1.07; 95% CI: 0.88–1.34), ASD (HR: 1.08; 95% CI: 0.88–1.33) or FAI (HR: 1.08; 95% CI: 0.88–1.34); neither were there any associations in the multivariable models. Furthermore, we did not detect any consistent associations between CVD mortality and TT (HR: 1.07; 95% CI: 0.81–1.40), SHBG (HR: 0.81; 95% CI: 0.59–1.12), and ASD (HR: 1.08; 95% CI: 0.77–1.52), fT (HR: 1.35; 95% CI: 0.96–1.91) and FAI (HR: 1.40; 95% CI: 0.98–1.99) (**Table 5**) in either age-adjusted models or multivariable models. After repeating all analyses with the second multivariable regression model, the overall findings, including level of significance, remained unchanged. Stratification for menopausal status, use of oral contraceptives, or use of hormone replacement therapy did not substantially alter the calculated estimates (see Supplementary Tables 1 and 2).

4. Discussion

In the present longitudinal, population-based study, we did not detect any consistent associations between sex hormone concentrations and CVD in age- and multivariable-adjusted analyses. SHBG was inversely and fT was positively associated with prevalent CVD only in the age-adjusted model, and the associations did not persist after multivariable adjustment. None of the sex hormones analyzed were associated with long-term all-cause mortality.

The present findings add to the conflicting results from observational studies concerning sex hormone concentrations and subclinical and clinical CVD in women. For example, the population-based Cardiovascular Health Study reported a J-shaped association between high serum TT and increased prevalence of CVD risk factors in 344 elderly women [6]. Likewise, a community-based study among 3297 women reported a strong cross-sectional association between low levels of SHBG and FAI and risk factors for CVD [7,20]. In contrast, though, a cross-sectional study among 101 pre- and post-menopausal women suggested that DHEAS and androgen levels in women are correlated with a lower risk of prevalent carotid artery sclerosis [21].

Similarly, the influence of androgens on incident CVD in women is far from well understood. For example, a 20-year follow-up of 1629 women from the population-based CARDIA study found that

SHBG, but not testosterone, was inversely associated with markers of incident subclinical CVD [22]. Additionally, a case-control study of 200 post-menopausal women over 3-year follow-up found that women with lower SHBG levels and a higher FAI were more likely to develop CVD, although these associations appeared to be dependent on diabetes, hypertension and BMI [5].

Other studies have investigated the effects of hyper androgenemia and polycystic ovary syndrome (PCOS) on cardiovascular outcome. Women with PCOS had more risk factors for CVD [23] and higher rates of subclinical CVD [24]. Additionally, a patient-based case-control study with 130 pre-menopausal women reported an inverse association between SHBG and DHEAS levels with subclinical CVD [25]. Many of these previous studies are limited by their cross-sectional design [23,25], or small numbers of women with diagnosed PCOS [24,25]. However, we were not able to replicate these previous analyses, since we did not assess PCOS in our cohort.

The comparatively young age and the mainly pre-menopausal status of our cohort may have reduced the study's potential to detect an association between sex hormones and CVD. In contrast, other studies have found that the use of combined oral contraceptives decreases circulating levels of TT and fT and increases SHBG concentrations [26]. Also, oral contraceptives are known to increase CVD risk among users with pre-existing CV risk factors, whereas carefully screened patients are not at increased risk of incident CVD from the use of oral contraceptives [27].

Previous studies investigating the potential association between sex hormones in women and all-cause mortality have yielded similarly conflicting results. A small four-year follow-up study among 97 elderly female patients at a care facility found no correlation between all-cause mortality and TT, but there was an association with low DHEAS concentrations [28]. Similarly, previous long-term follow-up studies [8,9] were not able to detect any associations between sex hormones and all-cause mortality. Other studies have shown that rates of all-cause and CVD-specific mortality are not elevated in women using oral contraceptives [29]. Given these contradictory results, the potential association between sex hormones in women in the general population and incident CVD and mortality risk remains to be elucidated.

The following limitations may help to explain the divergent findings of previous studies. First, the default immunoassay-based sex hormone measurements applied in previous studies are imprecise. A comparative study [30] has shown that the measurement of sex hormones via RIA, used in almost all previous studies [4–6,8,20–22,24,25,28], may have sufficient measurement error to yield biased risk associations. According to our knowledge, the present study is the first to measure sex hormones via LC/MS–MS for analyses of CVD and mortality risk. Second, the diverging results might be explained by differences in study samples. The previously reported associations were mostly based on selected patient samples [25,28], whereas the present study used a representative sample of women from the general population. Because the risk of CVD and mortality is related to age, the median age of 49.0 years in our study might explain the comparatively low number of CVD and mortality cases, which may lead to a lack of statistical power to detect potential risk associations between sex hormones and these outcomes. Also, genetic and environmental factors may have an impact on sex hormone concentrations and therefore lead to different results. In addition, the wide variety of definitions of CVD risk factors and CVD endpoints applied in previous studies hamper the comparability of their findings. Most studies have investigated the association between sex hormones and CVD and mortality only in post-menopausal women [3–6,8,28], but menopausal status, as well as age, may have a large influence on hormonal status. Furthermore, in the present study, since we did not assess the phase of the menstrual cycle at which blood samples were taken, we were not able to adjust for this potential source of bias in the

sex hormone measurements. Finally, our findings are not necessarily applicable to other ethnic groups or geographic regions, since the present study was conducted among white women residing in north-eastern Germany.

In conclusion, the present population-based longitudinal study showed no consistent association of sex hormones in women with incident CVD or mortality risk.

Author contributions

HW and RH conceived and designed the experiments. BGK and GB performed the sex hormone measurements. HK analyzed the data for this manuscript. HV, MD, MN contributed reagents/material/analysis tools. GS, HK and RH wrote the manuscript.

Competing interest

The authors have declared that no competing interests exist.

Ethical approval, consent or animal equivalent

The Ethics Committee of the University of Greifswald authorized the study protocol, which is consistent with the principles of the Declaration of Helsinki. All subjects participated only after written informed consent was obtained from each participant.

Disclosure

The authors have nothing to disclose.

Acknowledgements

SHIP is part of the Community Medicine Research net of the University of Greifswald, Germany, which is funded by the Federal Ministry of Education and Research (grants no. 01ZZ9603, 01ZZ0103, and 01ZZ0403), the Ministry of Cultural Affairs, as well as the Social Ministry of the Federal State of Mecklenburg–West Pomerania. This work is also part of the research project Greifswald Approach to Individualized Medicine (GANI_MED), and was supported by the DZHK (German Centre for Cardiovascular Research), and by the BMBF (German Ministry of Education and Research).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.maturitas.2015.08.009>.

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