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Assoziation inflammationsbezogener und elektrokardiografischer Parameter in der Allgemeinbevölkerung hinsichtlich kardiovaskulärer Endpunkte: Ergebnisse der CARLA-Studie

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Die Arbeit verfolgte die Zielsetzung, die Determination und Bedeutung inflammationsbezogener bzw. elektrokardiografischer Parameter hinsichtlich kardiovaskulär bedeutsamer Endpunkte zu untersuchen.

Hierbei lag der Fokus besonders auf der Relation inflammatorischer Biomarker (löslicher Typ 1 Rezeptor des Tumornekrosefaktors, C-reaktives Protein, Interleukin 6) mit anderen kardiovaskulär relevanten Größen, die sich nicht-invasiv messen lassen. Diese sind zum einen echokardiografische Parameter und die QT-Zeit, sowie die Herzfrequenzvariabilität als EKG-bezogene Größen, wobei letztere maßgeblich von dem autonomen Nervensystem bestimmt wird.

Als Datengrundlage diente die bevölkerungsbezogene CARLA-Studie, die ihr Studienkollektiv aus der älteren Allgemeinbevölkerung (45 bis 83 Jahre) in dem Raum Halle (Saale) rekrutierte und zum Zeitpunkt der Basisuntersuchung von 2002 bis 2006 1779 Teilnehmer umfasste.

Personen mit einem geringen Bildungsniveau zeigten eine erhöhte Konzentration des löslichen TNF-Rezeptors bzw. des C-reaktiven Proteins im Vergleich zu Personen mit einem höheren Bildungsgrad. Eine ähnliche Beziehung konnte für echokardiografische Parameter im Sinne einer erhöhten Herzmasse bei Personen mit geringer Bildung gezeigt werden.

Weiterhin zeigte sich eine positive Querschnittsassoziation des löslichen TNF-Rezeptors mit echokardiografischen Parametern der linksventrikulären Hypertrophie. Ebenfalls war dieser mit einer verlängerten QT-Zeit bei Frauen assoziiert. In dem Studienkollektiv war die Vorhersagekraft der QT-Zeit bezüglich der Mortalität von der linksventrikulären Masse abhängig. Hier zeigte sich eine schwächere Assoziation mit diesem Endpunkt bei steigender Herzmasse. Bezüglich der Herzfrequenzvariabilität fand sich eine Interaktion des Rezeptors mit Parametern des parasympathischen Nervensystems, wenn beide mit der Mortalität assoziiert wurden.

Schlussfolgernd ist der lösliche TNF-Rezeptor ein potentieller starker Prädiktor für kardiovaskuläre Endpunkte in der Allgemeinbevölkerung. Enge Verbindungen und Interaktionen mit etablierten Risikofaktoren unterstreichen den weiteren Forschungsbedarf.

Bibliografische Angaben

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Inhaltsverzeichnis

Abbildungen

Tabellen

Abkürzungen

BMI	Body-Mass-Index
CARLA	CARdiovascular Disease, Living and Ageing in Halle
CDE	Controlled Direct Effect, CDE (kontrollierter direkter Effekt)
CRP	C-reaktives Protein
DAG	Directed Acyclic Graph (gerichteter azyklischer Graph)
HDL	High Density Lipoprotein (Lipoprotein hoher Dichte)
HR	Hazard Ratio (Gefahrenquote)
HRV	Heart Rate Variability (Herzfrequenzvariabilität)
IDI	Integrated Discrimination Index (Integrierter Unterscheidungsindex)
IL-6	Interleukin-6
KI	Konfidenzintervall
LIFE	Losartan Intervention For Endpoint Reduction
LVH	linksventrikuläre Hypertrophie
NIE	Natural Indirect Effect (natürlicher indirekter Effekt)
NT-proBNP	N-Terminal pro-Brain Natriuretic Peptide
OR	Odds Ratio
RERI	Relative Excess Risk due to Interaction (durch Wechselwirkungen bedingtes verhältnismäßig hohes Risiko)
sICAM-1	Soluble Intercellular Adhesion Molecule-1 (lösliches intrazelluläres Adhäsionsmolekül Typ 1)
sTNF-R1	Soluble Tumor Necrosis Factor Receptor 1 (löslicher TNF-Rezeptor 1)
sTNF-R2	Soluble Tumor Necrosis Factor Receptor 1 (löslicher TNF-Rezeptor 2)
TACE	TNF Alpha Converting Enzyme
TE	Total Effect (Gesamteffekt)
WHR	Waist-to-Hip Ratio (Taillen-Hüft-Verhältnis)
WHtR	Waist-to-Height Ratio (Taille-Körpergröße-Verhältnis)

1 Einführung

1.1 Epidemiologische Studien

Seit dem Start der Framingham-Heart-Studie im Jahr 1948 (Mahmood *et al.* 2014) wurden zahlreiche bevölkerungsbasierte prospektive Kohortenstudien initiiert. In Deutschland ist an dieser Stelle allen voran die NAKO-Gesundheitsstudie (German National Cohort Consortium 2014) zu nennen. Diese große Kohortenstudie erstreckt sich über ganz Deutschland, erfasst ein umfassendes Untersuchungsprotokoll und wird ein Studienkollektiv von 100 000 Frauen und ebenso vielen Männern einschließen, wobei sich der Altersbereich von 20 bis 69 Jahren erstreckt (Abbildung 1).

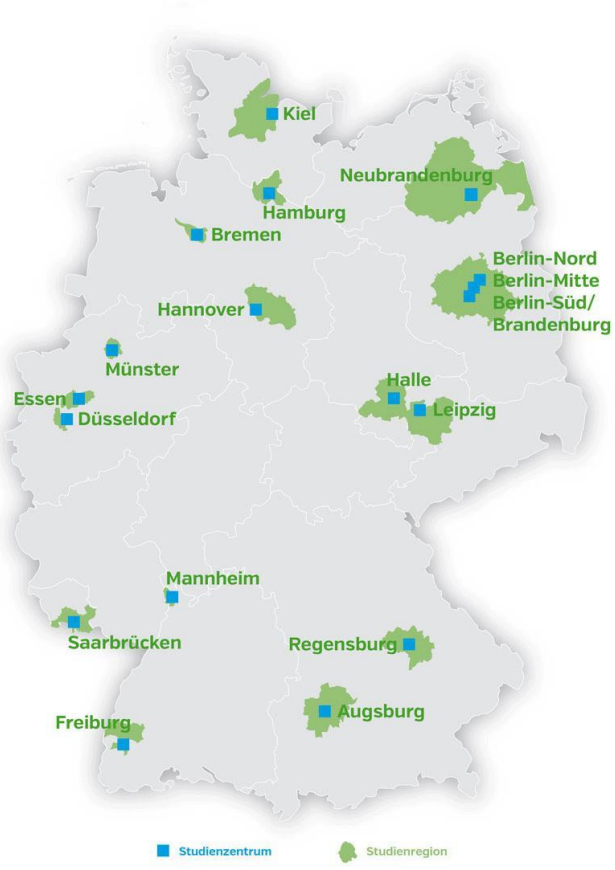

Abbildung 1: Studienzentren und Studienregionen im Rahmen der NAKO Gesundheitsstudie (German National Cohort Consortium, 2014)

In der Epidemiologie erfreuen sich diese Kohortenstudien großer Beliebtheit, da sie etliche Vorteile gegenüber anderen Formen der Beobachtungsstudie wie der Fall-Kontroll-Studie bieten. Im Zuge der Basisuntersuchung liegt der besondere Wert dieser Form der Beobachtungsstudie in der Möglichkeit, Größen standardisiert zu erheben. Darüber hinaus kann der prädiktive Wert einer ganzen Reihe von Variablen bei Betrachtung multipler Endpunkte in einem definierten Kollektiv der Bevölkerung getestet werden. Im Gegensatz zu den meisten retrospektiven Untersuchungen lassen sich hier Risiken unmittelbar berechnen.

Eines der grundlegenden Anliegen der Epidemiologie ist es, die Prävalenz und Inzidenz häufiger Erkrankungen zu bestimmen. Indem eine Zufallsstichprobe gezogen wird, die die Zusammensetzung der Allgemeinbevölkerung widerspiegelt, kann hier direkt von der Prävalenz bzw. Inzidenz in dieser Stichprobe auf die Gesamtbevölkerung, aus der sich die Studienkohorte rekrutiert, geschlossen werden. Darüber hinaus sind bevölkerungsbasierte Erhebungen ein tragender Pfeiler der Epidemiologie, da sie im Gegensatz zu klinischen Daten eine Risikoeinschätzung in einem Kollektiv erlauben, das frei von möglicher Verzerrung durch Krankheitsgeschehen ist. Dies war auch die Motivation der Initiatoren der Framingham-Studie, ein Kollektiv zu wählen, das frei von kardialen Erkrankungen war (Mahmood *et al.* 2014).

Es war dieser Studientyp, der es erstmals erlaubte, Risikofaktoren für häufige Zivilisationskrankheiten zu bestimmen. Ein berühmtes Beispiel stellt die Identifikation des Cholesterins als Risikofaktor für die koronare Herzkrankheit dar. In der Framingham-Studie wurden die ersten Ergebnisse zur inversen Beziehung zwischen dem High Density Lipoprotein (HDL) und dem Auftreten der koronaren Herzkrankheit im Jahr 1977 veröffentlicht (Gordon *et al.* 1977). Dass ein chronisches Vorhofflimmern ein Risiko für einen zerebralen Schlaganfall darstellt, ist heute hinlänglich bekannt. Die Evidenz für diese Beziehung stammte ebenfalls aus der Framingham-Studie, als Wolf et al. 1978 (Wolf *et al.* 1978) ein mehr als fünffach erhöhtes Risiko für das Auftreten eines Schlaganfalls bei Vorliegen des chronischen Vorhofflimmerns berichteten. Diese wenigen Beispiele zeigen, dass prospektive Kohortenstudien wichtige Beiträge zum Verständnis der Entstehung von Krankheiten und der individuellen Risikobewertung leisten. Das Verständnis dieser Zusammenhänge erlaubte dann im weiteren Verlauf pharmakologische Forschungen, die in die Entwicklung wirksamer Medikamente, etwa der Statine, die mittlerweile ein Eckpfeiler in der kardiologischen Prävention für Personen mit hohem Hochrisikoprofil darstellen, mündeten (Knopf *et al.* 2017).

Es ist nun die Aufgabe moderner epidemiologischer Studien, diese wissenschaftliche Tradition fortzuführen und dabei neue Risikofaktoren zu identifizieren bzw. ätiologische Relationen zwischen diesen und klinischen Endpunkten aufzuzeigen. Aktuelle Veränderungen des demografischen und medizinischen Umfelds sollen sich in solchen modernen Studien niederschlagen. Dabei stellt es eine besondere Herausforderung dar, Populationen mit erhöhtem Risiko für Erkrankungen mit hohem primären Präventionsbedarf zu betrachten. Für Ostdeutschland wurde eine erhöhte Prävalenz kardialer Erkrankungen berichtet (Robert-Koch-Institut 2011). In der Gesundheitsberichterstattung des Bundes publizierte Daten zeigen für die Gebiete der ehemaligen DDR einen sprunghaften Anstieg der verlorenen Lebensjahre pro 100 000 Individuen der Bevölkerung auf über 7000 bei Personen unter 65 Jahren – ein deutlich höherer Wert als in der Bevölkerung in den alten Bundesländern. In allen Gebieten zeichnete sich im weiteren Verlauf eine Abnahme der verlorenen Lebensjahre bis zum Jahr 2008 ab. Die nachteilige Gesundheitslage in den neuen Bundesländern bleibt aber weiterhin bestehen. Die Übersicht unterstreicht das hohe Risikoprofil dieser Bevölkerungsgruppe (Grigoriev & Pechholdová 2017), was sie besonders relevant für Beobachtungsstudien macht. Im Detail zeigt die Bevölkerung der neuen Bundesländer in allen Altersgruppen eine Übersterblichkeit gegenüber der Bevölkerung der alten Bundesländer. Werden die Jahre 1991/1993 gegenüber den Jahren 2006/2008 miteinander verglichen, so ist eine deutliche Abnahme dieser nachteiligen Sterblichkeitssituation zu beobachten, wenngleich diese Übersterblichkeit weiterhin in allen Altersgruppen bestehen bleibt. Über das Feld kardialer Erkrankungen hinaus findet sich eine erhöhte Sterblichkeit durch onkologische Erkrankungen in der Bevölkerung der neuen Bundesländer (Grigoriev & Pechholdová 2017; Medenwald *et al.* 2017b).

Im Zuge eines demografischen Wandels zeigt gleichzeitig die ältere Allgemeinbevölkerung die stärkste Relevanz für epidemiologische Fragestellungen im Kontext bevölkerungsbasierter Krankheiten. In Deutschland waren im Jahr 2015 kardiovaskuläre Erkrankungen die führende Todesursache. Im Detail waren 172 637 Todesfälle auf einen akuten Myokardinfarkt, die chronische ischämische Herzkrankheit oder eine Herzinsuffizienz zurückzuführen. Dies entspricht einem relativen Anteil der chronischen ischämischen Herzkrankheit von 8,2 % an allen Todesfällen bzw. einem Anteil von 5,3 % des akuten Myokardinfarktes und 5,1 % der chronischen Herzinsuffizienz an allen Todesursachen. Diese Entwicklung verdeutlicht noch einmal die außerordentliche Relevanz der älteren Allgemeinbevölkerung für die epidemiologische Forschung.

1.2 Chronische Inflammation im Kontext kardiovaskulärer Erkrankungen

Neben den bekannten Risikofaktoren für kardiovaskuläre Mortalität und Morbidität stellen Entzündungsparameter neue Biomarker dar, die weitere Informationen zum Risikoassessment aufzeigen können. Mehrere Studien zeigten eine breite Evidenz für einen prädiktiven Wert chronischer Inflammation anhand bevölkerungsbasierter Kollektive (Li & Fang 2004; Pai *et al.* 2004). Eine Mehrheit der historischen Studien konzentrierte sich dabei auf das C-reaktive Protein in solchen Kohorten (Li & Fang 2004; Pai *et al.* 2004). Dieses Protein wird im Rahmen manifester Entzündungsprozesse gebildet und lässt sich leicht im peripheren Serum nachweisen (Ridker *et al.* 2000; Kuo *et al.* 2005). In einer der ersten Arbeiten zu diesem Thema untersuchten Ridker et al. (Ridker *et al.* 2000) ein großes Kollektiv von 28 263 Frauen, die sich zum größten Teil aus der Women's Health Study rekrutierten (71 %). Neben dem genannten C-reaktiven Protein (CRP) untersuchten die Autoren im Rahmen dieser Nested-Case-Control-Studie (eine Fall-Kontroll-Studie, eingebettet in eine prospektive Kohortenstudie) weitere Inflammationsparameter (Serum-Amyloid A, Interleukin-6 [IL-6], lösliches intrazelluläres Adhäsionsmolekül Typ 1 [sICAM-1]). Diese Parameter wurden auf ihren prädiktiven Wert für kardiovaskuläre Ereignisse (Tod durch koronare Herzkrankheit, nicht tödlicher Myokardinfarkt, Schlaganfall oder die Notwendigkeit einer koronaren Revaskularisation) getestet. Es zeigte sich, dass aus diesen erhobenen Parametern das C-reaktive Protein die stärkste Assoziation mit der primären Zielgröße zeigte. Weitere Studien setzten das CRP mit dem Auftreten kognitiver Defizite bzw. Depression in Verbindung und fanden ein erhöhtes Risiko bei einem Anstieg des CRP für die genannten Endpunkte (Kuo *et al.* 2005), was die ubiquitäre Bedeutung von Entzündungsreaktionen unterstreicht.

In den letzten Jahren konzentrierten sich modernere Studien auf weitere Inflammationsparameter, die eine möglicherweise zusätzliche Vorhersagekraft über das CRP hinaus aufweisen. In einer richtungsweisenden Arbeit untersuchten Pai et al. (Pai *et al.* 2004) die Assoziation verschiedener Marker (CRP, IL-6, Soluble Tumor Necrosis Factor Receptor 1 [sTNF-R1] und Soluble Tumor Necrosis Factor Receptor 2 [sTNF-R2]) der chronischen Entzündung anhand zweier großer Kollektive. Grundlage der Untersuchung bildete die Nurses' Health Study, die allein Teilnehmerinnen umfasste. Männer wurden aus der Health Professionals Follow-up Study rekrutiert. Die Autoren verfolgten dabei einen Nested-Control-Ansatz. Die grundlegende Idee dieser Methodik ist es, aus einem größeren Studienkollektiv Fälle gemäß dem primären Outcome zu identifizieren. Diesen werden dann in einem zweiten Schritt entsprechende Kontrollen aus derselben Kohorte zugeordnet, wobei im konkreten Fall ein Risk-Set Sampling angewandt wurde, das es erlaubt, Inzidenzraten zu berechnen (Rodrigues & Kirkwood 1990). Im Falle der Studie

der Forscher Pai et al. wurden Fälle mit nicht tödlichem myokardialen Infarkt bzw. Todesfälle auf Grund der koronaren Herzkrankheit betrachtet. Hierbei zeigte sich für den Vergleich der Probandengruppe mit einer Blutkonzentration des CRP über 3,0 mg/l im Vergleich zur Referenz (< 1 mg/l) eine Assoziation mit dem Fall-Kontroll-Status allein für das CRP, wenn mögliche Confounder in den Modellen berücksichtigt wurden (1,68; 95-%-Konfidenzintervall [KI]: 1,18–2,38).

Im Gegensatz dazu zeigten Ueland et al. (Ueland *et al.* 2005) den prädiktiven Wert des löslichen TNF-Rezeptors in einem Kollektiv von Patienten, die sich in der Akutphase nach einem Myokardinfarkt befanden. Nach Adjustierung für das N-Terminale pro-Brain Natriuretic Peptide (NT-proBNP) stellte sich ein risikoerhöhender Effekt für die Gesamt- und kardiovaskuläre Mortalität durch den sTNF-R1 mit einem Hazard Ratio von 6,2 (95-%-KI: 1,9–20,7) bzw. 5,5 (95-%-KI: 1,6–18,3) dar, was für weitere Zytokine wie das Interleukin-6 nicht berichtet werden konnte.

Die löslichen TNF-Rezeptoren werden als membranständige Proteine in zwei Formen exprimiert (sTNF-R1 und sTNF-R2). Beide sind stark miteinander korreliert, sodass es zur Beantwortung der meist empirischen Fragestellungen in den meisten Fällen ausreicht, eine Form zu bestimmen (Campbell *et al.* 2003; Carlsson *et al.* 2014). Im Gegensatz zu vielen anderen Entzündungsmarkern besitzt der lösliche TNF-alpha-Rezeptor eine starke zeitliche Stabilität, was ihn besonders attraktiv für Analysen im Rahmen von Beobachtungsstudien erscheinen lässt (Carpena *et al.* 2012), die nur singuläre Messungen mit großen zeitlichen Abständen als temporär umschriebene Follow-up-Untersuchungen erlauben. Im weiteren Fortgang der Arbeit werden die löslichen TNF-Rezeptoren eine zentrale Rolle als Biomarker für kardiovaskuläre Erkrankungen einnehmen. Nach Bildung des TNF-alpha als membranständiges Protein wird dieses Molekül durch das sogenannte TNF Alpha Converting Enzyme (TACE) von der Zelloberfläche abgespalten (Campbell *et al.* 2003). In der Zielzelle werden dann weitere Reaktionen angestoßen, die die zelluläre Translation und Transkription steuern. Damit kann es seine physiologische Wirkung an der Zielstruktur entfalten. Diese Zusammenhänge sind bildhaft in *Abbildung 2* dargestellt.

Die Rolle des TNF in der kardialen Pathophysiologie wird nicht allein durch die unmittelbare Rezeptorfunktion bestimmt. In einer frühen Arbeit wies Aderka (Aderka 1996) auf die regulatorische Funktion des dissoziierten Rezeptors hin. Hintergrund ist der Prozess der Dissoziation des Rezeptors von seiner intrazellulären Domäne, die sowohl durch das TNF-alpha selbst als auch durch weitere Zytokine wie das Interleukin-6 hervorgerufen werden kann. Der lösliche Rezeptor wiederum kann nun an den inflammatorisch wirkenden TNF-alpha binden und diesen somit neutralisieren.

Aus epidemiologischer Sicht stellt sich somit die Frage, ob die TNF-Rezeptoren eher als protektive oder als Risikofaktoren einzuschätzen sind. Werden die Effektschätzer aus der Literatur betrachtet, so deutet sich eine Dominanz als risikoerhöhender Biomarker an (Ueland *et al.* 2005; Valgimigli *et al.* 2005; Medenwald *et al.* 2017a).

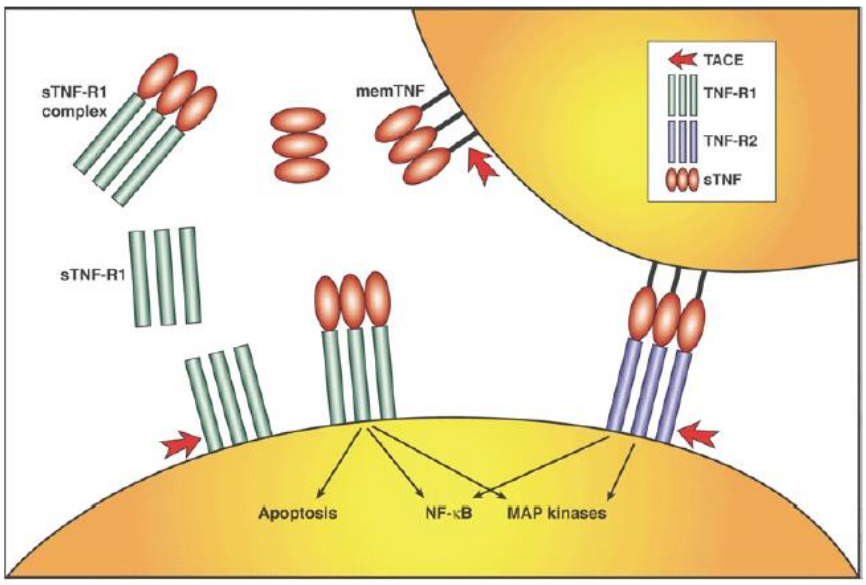

Abbildung 2: Interaktion von löslichem Tumornekrosefaktor-alpha (soluble TNF: sTNF) und membranständigem Tumornekrosefaktor (memTNF) mit TNF-Rezeptoren (TNF-R1 und TNF-R2). Das TNF Alpha Converting Enzyme (roter Pfeil, TNF-konvertierendes Enzym) ist dabei für den Dissoziationsprozess entscheidend (Campbell et al. 2003).

Ein weiterer Parameter, der in zahlreichen Studien (Ridker *et al.* 2000; Pai *et al.* 2004; Lacey *et al.* 2017) auf seine Assoziation mit klinischen Outcomes hin untersucht wurde, ist das Interleukin-6. Dieses Interleukin nimmt zahlreiche Funktionen im Rahmen der Immunantwort wahr. Für die bloße Betrachtung des IL-6 als Biomarker im epidemiologischen Setting ist die Fähigkeit des IL-6, eine Synthese des CRP zu induzieren, besonders relevant (Ridker 2014; Lacey *et al.* 2017). Auf zellulärer Ebene spielen Makrophagen, die sich an die vaskuläre Intima anlagern, eine Schlüsselrolle. Sie sezernieren als Schaumzellen ihrerseits proinflammatorische Proteine wie das IL-6 oder TNF-alpha, die wiederum Adhäsionsmoleküle bilden und damit eine weitere Kondensation inflammatorischer Zellen bewirken (Kriszbacher *et al.* 2005; Weber & Noels 2011). Final münden diese Prozesse in die Formation atherosklerotischer Plaques, die dann koronare Gefäße okkludieren können (Antoniades *et al.* 2009).

Ähnlich wie im Fall des TNF-alpha bindet das IL-6 an einen zweidomänigen Rezeptor (Mauer *et al.* 2015). Eine erhöhte Produktion des IL-6 war mit einer vermehrten Bildung

des zellulären Rezeptors assoziiert. Die Ursprungsorte einer chronischen Inflammation, die durch IL-6 vermittelt werden, sind vielfältig. Unter den möglichen Ursprungsorten nimmt das viszerale Fettgewebe als stoffwechselaktives Organ eine Schlüsselstellung ein (Mauer *et al.* 2014). Diese Aussage trifft nicht nur für das IL-6, sondern für die chronische Entzündungsreaktion allgemein zu und wird im Verlauf vorliegender Arbeit noch weiter diskutiert werden.

In einer gepoolten Analyse aus zwei großen Kohortenstudien untersuchten Danesh et al. die Assoziation des IL-6 mit der koronaren Herzkrankheit mit einem Fall-Kontroll-Ansatz (Danesh *et al.* 2008) und fanden eine deutliche Assoziation mit einem Odds-Ratio von 1,61 (95-%-KI: 1,42–1,83). Weitere Arbeiten fanden ähnliche Effekte im Sinne einer positiven Assoziation des IL-6 mit inzidenter koronarer Herzerkrankung (Kaptoge *et al.* 2014).

1.3 Elektrophysiologische Parameter: Herzfrequenzvariabilität als elektrophysiologischer Parameter des autonomen Nervensystems

Die elektrokardiologische Untersuchung erlaubt es, eine ganze Reihe an Größen zu bestimmen, die Auskunft über kardiale Risikokonstellationen geben sollen. Im Rahmen dieser Arbeit soll das Augenmerk auf die QT-Zeit und die Herzfrequenzvariabilität (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology 1996) gerichtet werden. Für eine eingehendere Besprechung des Wertes kardialer Indizes, die mit einer ventrikulären Hypertrophie assoziiert sein sollen, etwa dem Sokolow-Lyon-Index, sei hier auf andere Arbeiten im Rahmen der CARLA-Studie verwiesen (Schröder *et al.* 2015). Zusammenfassend wurde hier gezeigt, dass der Sokolow-Lyon-Index nur schwach mit der linksventrikulären Hypertrophie assoziiert ist (Schröder *et al.* 2015). Diese schwache Vorhersagekraft wiesen auch andere Arbeiten (Schillaci *et al.* 2012; Cuspidi *et al.* 2014; Czosek *et al.* 2014) in verschiedenen Kollektiven nach, was den Wert dieser Indizes allgemein infrage stellt.

Als weiterer Parameter der linksventrikulären Hypertrophie wurde die QT-Zeit untersucht. Dieses Zeitintervall im EKG ist definiert als die Zeit zwischen dem Beginn des QRS-Komplexes und dem Ende der T-Welle. Im klinischen Kontext ist eine Verlängerung dieser Strecke mit dem Auftreten einer Torsade-de-Pointes-Tachykardie in Verbindung gebracht worden (Elming *et al.* 2002), die selbst wiederum in einen plötzlichen Herztod münden kann. Frauen haben im Vergleich zu Männern für diese Form der Tachykardie ein erhöhtes Risiko. Der Wert der QT-Zeit ist stark abhängig von der Herzfrequenz, was die Interpretation erheblich beeinträchtigt. Zur Korrektur des Wertes je nach Herzfrequenz wurden verschiedene Verfahren vorgeschlagen. Ein schon im Jahr 1920

veröffentlichtes Verfahren stellt die Korrektur nach Bazett (Bazett 1997) dar, die folgende Formel zur Berechnung der korrigierten QT-Zeit (QTcB) benutzt:

$$QTcB = \frac{QT}{\sqrt{RR}}$$

Allerdings zeigte sich, dass dieses Verfahren die QT-Zeit nicht vollständig für die Herzfrequenz korrigiert und eine Korrelation zwischen der korrigierten QT-Zeit (QTc) bestehen bleibt. Eine weitere Möglichkeit stellt die Korrektur nach Fridericia dar (Fridericia 2003), die weniger anfällig für eine Beeinflussung durch die Herzfrequenz sein soll und wie folgt berechnet wird:

$$QTcF = \frac{QT}{\sqrt[3]{RR}}$$

In einem jüngsten Vergleich beider Ansätze fanden Vandenberk et al. (Vandenberk *et al.* 2016) eine Überlegenheit der Korrektur nach Fridericia gegenüber weiteren Verfahren wie der Bazett-Formel, wenn das Kriterium der Mortalität nach 30 Tagen bzw. nach einem Jahr zugrunde gelegt wird.

Goldenberg et al. (Goldenberg *et al.* 2006) definierten für Frauen einen Schwellenwert für eine Herzfrequenz-korrigierte QT-Zeit von über 460 ms und bei Männern von über 450 ms als erhöht. Besondere Bedeutung erhält die QT-Zeit in einem klinischen Umfeld, da verschiedene Medikamente eine Verlängerung dieses Intervalls induzieren können (Woosley 31/01/2018).

Entzündungsreaktionen stehen nicht allein, sondern sind von dem ebenfalls ubiquitären autonomen Nervensystem beeinflusst. Dieses ist grundsätzlich schwierig in seiner Aktivität zu quantifizieren. Eine einfache Methode stellt die elektrokardiografische Bestimmung mittels der sogenannten Herzfrequenzvariabilität dar.

Im Allgemeinen gibt es die traditionell betrachteten linearen Parameter der Heart Rate Variability (HRV, Herzfrequenzvariabilität) und die in jüngerer Zeit eingeführten nicht linearen Parameter. Gemäß dem Verfahren, das für ihre Berechnung verwendet wird, können unter den erstgenannten Parametern (lineare HRV-Parameter) weitere Paramater der Zeitdomäne und der Frequenzdomäne zusätzlich abgegrenzt werden (Billman 2011).

Obwohl die HRV traditionell aus dem 24-h-Elektrokardiogramm berechnet wurde, wurden während des letzten Jahrzehnts Kurzzeitaufnahmen eingeführt. Hier zeigte sich,

dass Werte aus Kurzzeitmessungen über Monate stabil sind, wenn sie unter standardisierten Bedingungen aufgezeichnet wurden (Sinnreich *et al.* 1998).

Relevante HRV-Parameter sind in Tabelle 1 aufgelistet. Neben den genannten Parametern gibt es noch weitere Parameter wie beispielsweise die sehr niedrige Frequenzleistung. Sie erfordern jedoch aufgrund ihrer niedrigen Frequenz eine Langzeitaufzeichnung, die in der CARLA-Studie nicht realisiert wurde.

Tabelle 1: Übersicht relevanter Parameter der Herzfrequenzvariabilität

Standard Deviation of Normal Interval (SDNN) [ms]	- Die allgemeinste Form der HRV-Messung - Standardabweichung normaler RR-Intervalle (Gesamtvariabilität) - Kann als Parameter des parasympathischen Systems interpretiert werden (Pumprla *et al.* 2002)
Root Mean Square Successive-Normal-Interval-Differences (RMSSNID) [ms]	- Quadratwurzel der durchschnittlichen quadratischen Differenz zwischen benachbarten RR-Intervallen - Parameter der parasympathischen Aktivität
Low frequency power (LF) [ms^2]	- Niederfrequenzleistung ist die Dichte des Leistungsspektrums zwischen 0,04 und 0,15 Hz (Rajendra Acharya *et al.* 2006) - Vorwiegend vom sympathischen autonomen System beeinflusst (umstritten)
High frequency power (HF) [ms^2]	- HF liegt zwischen 0,15 und 0,4 Hz und wird als Parameter des parasympathischen Systems interpretiert (Rajendra Acharya *et al.* 2006)
Verhältnis aus LF und HF (LF/HF)	- Maß für das Gleichgewicht zwischen beiden Zweigen des autonomen Systems (Rajendra Acharya *et al.* 2006)

Im Kontext kardiovaskulärer Erkrankungen gilt es, die Bedeutung solcher Inflammationsparameter als Ausdruck einer kardialen Manifestation chronischer Entzündung über die bloße Assoziation mit Mortalitätsendpunkten hinaus zu untersuchen.

Auf empirischer Ebene ist diese Beziehung zwischen Entzündung und autonomem Nervensystem im Zusammenhang mit einer dritten Größe (Endpunkt) als Interaktion beschreibbar. Eng verbunden mit der biometrischen Erhebung einer Interaktion ist das Problem unterschiedlicher Skalen. Interaktionen können epidemiologisch auf einer multiplikativen und additiven Ebene gemessen werden. Rothman argumentierte für die Anwendung der additiven Skala bei der Bewertung biologischer Prozesse im Sinnes eines

Sufficient-Cause-Modells (Rothman *et al.* 2008). Um Ereigniszeitmodelle und damit Endpunkte des Überlebens analysieren zu können, führte Knol et al. eine Methode zur Berechnung von Interaktionen auf additiven Skalen ein. Zur Abgrenzung von multiplikativen Verfahren wird diese Form der Interaktion bei Knol et al. als „Relative Excess Risk due to Interaction“, kurz RERI (Knol *et al.* 2011), bezeichnet. Aus den Effektschätzern (Betas) eines hinlänglichen Cox-Regressionsmodells wird der RERI mit nachfolgend genannter Formel berechnet:

$$RERI = e^{\widehat{\beta_1}+\widehat{\beta_2}+\widehat{\beta_3}} - e^{\widehat{\beta_1}} - e^{\widehat{\beta_2}} + 1$$

1.4 Problematik chronischer Entzündung in epidemiologischen Studien

Bevölkerungsbasierte Studien zeigen besondere Anforderungen, die an die Bewertung des risikoprädiktiven Effekts neuer Biomarker zu stellen sind. Grundsätzlich stellt sich die Frage nach der zusätzlichen Information, die sie im Vergleich zu etablierten Risikofaktoren zum Risiko für kardiale Endpunkte liefern. In großen epidemiologischen Studien wurden Expositionen bzw. Faktoren, die z. B. durch das Ernährungsverhalten grundsätzlich beeinflussbar sind, in Relation zu kardialen Endpunkten untersucht (Pischon *et al.* 2008; Sharma *et al.* 2016).

Diese sind von bereits etablierten laborchemischen Biomarkern zu trennen. Unter diesen hat das NT-proBNP sowohl in einem klinischen als auch bevölkerungsbasierten Setting die größte Relevanz für die Assoziation mit kardiovaskulären Endpunkten erreicht (Taylor *et al.* 2014; Kara *et al.* 2015; Dietl *et al.* 2016).

Ein weiteres Problem ergibt sich aus der rechtsschiefen Verteilung der Inflammationsparameter, die nach unten durch die Null begrenzt werden. Bei der Anwendung gängiger statistischer Verfahren kann sich aus diesem Umstand das Problem verzerrter Effektschätzer entwickeln. Grundsätzlich stehen dem aber verschiedene Verfahren gegenüber, die es erlauben, die Modelle zu analysieren bzw. an die Schiefe der Verteilung anzupassen. Viele Arbeiten nutzen eine logarithmische Transformation der Parameter. Das vordringliche Problem bei diesem Ansatz stellt dabei die mit dieser Umskalierung einhergehende Transformation der Skalen dar. Das bedingt Schwierigkeiten bei der Interpretation der Effektschätzer, da diese nach erfolgter Logarithmierung auf einer multiplikativen Skala berechnet werden. Zur Illustration bietet sich an, sich eine Verdoppelung der Blutkonzentration des CRP bei einem initialen Wert von 2 mg/l auf 4 mg/l vorzustellen, die auf der multiplikativen Skala die gleiche Risikoerhöhung mit sich bringen würde

wie eine Erhöhung von 30 mg/l auf 60 mg/l. Das Problem der Verwendung einer additiven oder multiplikativen Skala wird durch Rothman (Rothman *et al.* 2008) und Knol et al. (Knol *et al.* 2011) eingehend erläutert. Beide argumentierten für die Nutzung der additiven Skala bei Fragestellungen, die primär auf biologische bzw. kausale Prozesse zielen, insbesondere bei der statistischen Untersuchung von Interaktionen.

Zur Bewertung des Einflusses einzelner Beobachtungen auf das Modell bei der linearen Regression stellt die „Cooks's Distance" (Demidenko & Stukel 2005) – ein einfaches Verfahren zur groben Abschätzung von Variablen mit starkem Einfluss bzw. zur Identifikation möglicher Ausreißer – dar, wie es bei schiefverteilten Größen (z. B. dem CRP) wahrscheinlich ist (wenige Probanden mit sehr hohen Werten). Als nicht parametrische Größe, die sich nicht statistisch testen lässt, muss ein Schwellenwert definiert werden. In der Literatur wurde wiederholt eine Cook's Distance über eins als Cut-off für Beobachtungen mit starkem Einfluss definiert (Demidenko & Stukel 2005).

2 Zielsetzung

Die hier vorgelegte Arbeit untersucht die folgenden Fragestellungen:

(1) Wird nach möglichen Ursachen einer erhöhten Entzündungslast gesucht, erscheint besonders das viszerale Fettgewebe als ein Ort starker chronischer Entzündungsaktivität. Gleichzeitig zeigten frühere Arbeiten (Knopf *et al.* 1999), dass Übergewicht eine erhöhte Prävalenz in Bevölkerungsschichten mit geringem Bildungsniveau hat. Diese hypothetische kausale Kette soll analysiert werden. Die Hypothese lautet in diesem Zusammenhang, dass zum einen Personen mit einem geringeren Bildungsniveau ein höheres Entzündungsniveau zeigen. Eine zu bestimmende Proportion dieses Effekts wird dabei über anthropometrische Parameter vermittelt, die mit Übergewicht in Verbindung stehen. Als Formel dienen also die anthropometrischen Parameter als Mediatoren des Effekts des Bildungsniveaus auf den Grad der Entzündung.

(2) Eine analoge Argumentation ist für herzstrukturelle Parameter zu führen. In Analogie zur Fragestellung (1) soll ebenfalls die Assoziation des Bildungsniveaus mit echokardiografischen Parametern der linksventrikulären Masse überprüft werden. Auch hier lässt sich dieser Effekt durch eine Mediatoranalyse quantifizieren.

(3) Inflammationsparameter sowie echokardio- und elektrokardiografische Parameter der linksventrikulären Hypertrophie sollen auf ihre prospektive Assoziation getestet werden. Im Detail bedeutet dies, dass die Beziehung zwischen Inflammationsparametern (CRP, IL-6, sTNF-R1) und echokardiografischen Parametern sowohl durch Querschnitts- als auch durch eine Analyse im Längsschnitt untersucht werden soll. Letztere ist begrenzt durch die fehlenden Werte für das IL-6 und den sTNF-R1 im zweiten Follow-up der CARLA-Studie. Für die QT-Zeit soll ein analoges Vorgehen gewählt werden. Auf eine tiefere Besprechung der prospektiven Analysen soll hier verzichtet werden, da zum Zeitpunkt der Durchführung dieser Erhebung der sTNF-R1 nur in der Basiserhebung bestimmt worden war und damit prospektive Untersuchungen nur sehr eingeschränkt möglich sind, insbesondere Analysen der Veränderung des sTNF-R1.

(4) Aufgrund ihrer engen Verbindung sollen QT-Zeit und echokardiografische Parameter, hier besonders die linksventrikuläre Masse, eingehender untersucht werden. Wie in der Einleitung erwähnt, zeigten frühere Arbeiten eine enge Beziehung zwischen QT-Zeit und Herzmasse. Unter Berücksichtigung dieses Phänomens ist zu argumentieren, dass der Effekt der QT-Zeit als entscheidender Risikofaktor für den plötzlichen Herztod von dem Grad der linksventrikulären Hypertrophie beeinflusst wird. Im epidemiologischen Kontext stellt sich bei einer solchen Konstellation die Frage nach einer möglichen Interaktion zwischen beiden Parametern, gemessen an einem harten Zielkriterium, etwa der Gesamt- bzw. kardiovaskulären Mortalität.

(5) Der prädiktive Wert des sTNF-R1 für Mortalitätsendpunkte ist bisher nicht in einem populationsbezogenen Kontext untersucht worden. Analog zum CRP ist hier mit einem risikoerhöhenden Effekt des sTNF-R1 bei Betrachtung der kardiovaskulären oder Gesamtmortalität zu argumentieren.

(6) Die Interaktion des autonomen Nervensystems mit Inflammationsparametern soll auf den Endpunkt der kardialen und allgemeinen Mortalität hin analysiert werden. Um die Aktivität des autonomen Nervensystems zu quantifizieren, soll als nicht invasive Methode die Herzfrequenzvariabilität (HRV) angewandt werden.

3 Material und Methoden

3.2 Studienkollektiv

Die genannten Zielsetzungen sollen auf Grundlage der sogenannten „CARdiovascular Disease, Living and Ageing in Halle“-Studie (CARLA-Studie) verfolgt werden. Diese Kohorte ist aufgrund der Allokationsmethode repräsentativ für die Allgemeinbevölkerung der Stadt Halle im Alter von über 45 Jahren. Zum Zeitpunkt der Basisuntersuchung umfasste die CARLA-Kohorte 1779 Teilnehmer (967 Männer, 812 Frauen) im Alter zwischen 45 und 83 Jahren (Greiser *et al.* 2005). Die Basiserhebung fand zwischen Dezember 2002 und Januar 2006 statt. Die die relative Teilnahme nach erfolgter Einladung (Response) dieser Studie betrug nach Ausschluss aller Personen, die vor der Einladung verstorben oder weggezogen waren bzw. wegen Krankheit nicht teilnehmen konnten, zum Basiszeitpunkt 64 %. Insgesamt erfolgten zwei Follow-up-Untersuchungen, wobei das erste Follow-up zwischen den Jahren 2007 und 2010 durchgeführt wurde. Ein zweites Follow-up fand zwischen Juli 2012 und Oktober 2013 statt.

Die Studie wurde von der Ethikkommission der Medizinischen Fakultät der Martin-Luther-Universität Halle-Wittenberg und vom Landesdatenschutzbeauftragten des Landes Sachsen-Anhalt genehmigt und entspricht den in der Deklaration von Helsinki dargelegten Grundsätzen. Alle Teilnehmer gaben eine schriftliche Einverständniserklärung entsprechend einen „Informed Consent“ ab. Eine detaillierte Beschreibung der CARLA-Studie findet sich bei Greiser et al. (Greiser *et al.* 2005). Im Folgenden soll die Erhebung relevanter Kernparameter kurz umrissen werden.

Die CARLA-Studie reiht sich damit in eine ganze Reihe von bevölkerungsbasierten Kohortenstudien ein (Tabelle 2).

Tabelle 2: Übersicht der mit der CARLA-Studie vergleichbaren prospektiven Kohortenstudien in Deutschland

Studie	Altersbereich	Response-Rate
Heinz-Nixdorf-RECALL-Studie (Stang *et al.* 2005)	45–74 Jahre	56 %
Rotterdam-Studie (Ikram *et al.* 2017)	Cohort 1 (1990) and 2 (2000): > 55 years; Cohort 3: 45–54 (2008)	72 %
SHIP-Studie (Völzke 2012)	20–79 Jahre	69 %
CARLA-Studie (Greiser *et al.* 2005)	45–80 Jahre zum Zeitpunkt der Baseserhebung	64 %

Die genannten Studien zeichnen sich durch eine Erhebung echokardiografischer Parameter aus. Die Response-Rate der CARLA-Studie befindet sich auf einem ähnlichen Niveau wie die Response-Raten der genannten prospektiven Studien.

3.3 Statistische Verfahren

Um die im vorherigen Abschnitt genannten Forschungsfragen zu analysieren, wurden statistische bzw. epidemiologische Standardverfahren angewandt. Auf diese Methoden soll an dieser Stelle nicht vertiefend eingegangen, sondern stattdessen auf die entsprechenden Publikationen verwiesen werden.

Daneben kamen Verfahren der „Causal Inference"-Analysen zur Anwendung. Diese Verfahren beschrieb VanderWeele (VanderWeele 2015) eingehend. Einschränkend muss an dieser Stelle erwähnt werden, dass diese Methoden ursprünglich für randomisierte Studien entwickelt worden waren, die das Problem der Strukturungleichheit durch das Studiendesign vermeiden. Vor diesem Hintergrund müssen die Daten unter Berücksichtigung der folgenden Kriterien interpretiert werden (VanderWeele & Vansteelandt 2014). Diese Beziehung und die entsprechende Nomenklatur sind in *Abbildung 3* illustriert:

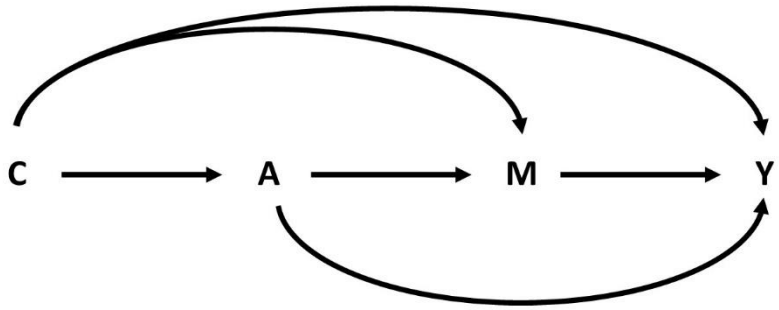

Abbildung 3: Angenommene kausale Beziehung nach Valeri und VanderWeele 2013

(1) *„conditional on C, there is no unmeasured confounding for the exposure-outcome relationship*
(2) *there is no unmeasured confounding for the mediator-outcome relationship*
(3) *conditional on C, there is no unmeasured confounding of the exposure-mediator relationship*
(4) *no effect L of exposure A that itself affects both M and Y, i.e. no effects of exposure A that confound the mediator-outcome relationship“*

Bei den Methoden zur Mediatoranalyse sollen an dieser Stelle nur die Grundlagen erläutert werden, die für ein Verständnis der Ergebnisse notwendig sind (Valeri & VanderWeele 2013; VanderWeele & Vansteelandt 2014; VanderWeele 2016). Der Mediatoranalyse liegen die Begriffe des Mediators, des Gesamteffekts (Total Effect, TE), des natürlichen indirekten Effekts (Natural Indirect Effect, NIE) sowie des direkten Effekts (Controlled Direct Effect, CDE) zugrunde. Zur Illustration der Effekte sei auf *Abbildung 3* und *Abbildung 4* verwiesen. Im Detail bezeichnet der Gesamteffekt die Summe aus dem NIE und CDE, wobei hierzu durch VanderWeele verschiedene Bedingungen formuliert wurden, die es einzuhalten gilt, damit solche Beziehungen ohne eine Effektverzerrung geschätzt werden können. Diese grundlegenden kausalen Relationen sind in *Abbildung 4* exemplarisch gezeigt. Eine andere Anwendung erfahren diese kausalen Modelle im Rahmen der Directed Acyclic Graphs (DAG), die es erlauben, mögliche Confounder anhand kausaler Pfade auszuwählen, und die ihm Rahmen dieser Arbeit angewandt wurden (Textor *et al.* 2011).

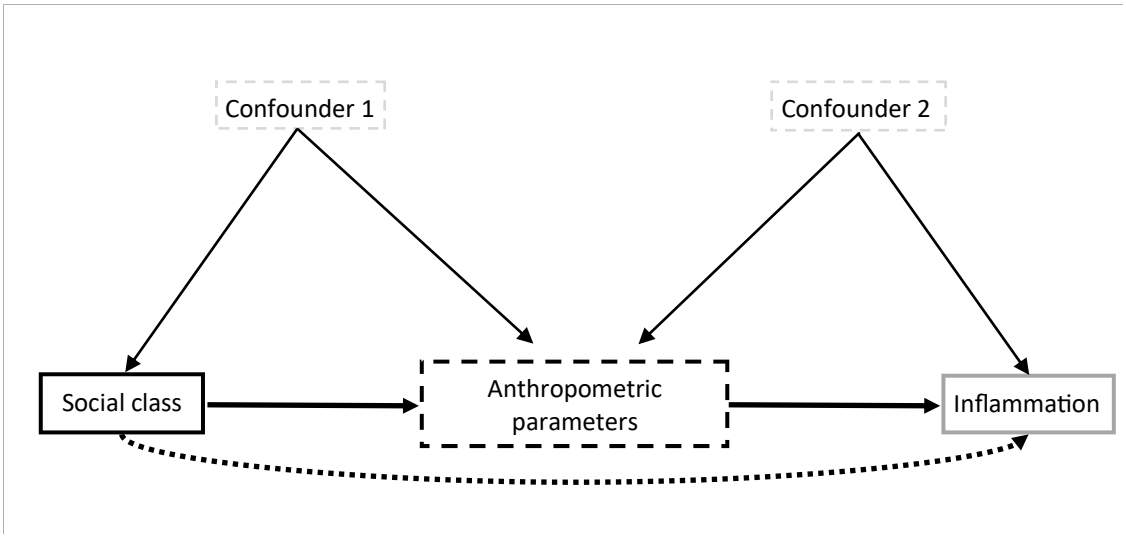

Abbildung 4: Kausale Beziehungen der Mediatoranalyse

Solide Linie: Natural Indirect Effect (NIE); gestrichelte Linie: Controlled Direct Effect (CDE)

4 Zusammenfassung der Ergebnisse

Die Determinanten für die Entzündungslast in der Bevölkerung zeigten eine starke Evidenz für den Einfluss des Bildungsniveaus als Surrogat des sozioökonomischen Status. Auf dem kausalen Pfad vom Bildungsniveau zum geschätzten Level der Inflammation liegen anthropometrische Größen. Bei der Analyse solcher Indizes zeigten sich die eingeschränkte Bedeutung des Verhältnisses des Taillenumfangs zum Hüftumfang (WHR) gegenüber anderen Größen wie dem BMI (Medenwald *et al.* 2015). Anthropometrische Parameter korrelierten mit allen Entzündungsparametern nach Berücksichtigung eventueller Confounder. Der BMI und das Verhältnis der Taille zur Körpergröße (Waist-to-Height Ratio: WHtR) waren starke Mediatoren der Beziehung zwischen Bildungsunterschieden des sTNF-R1 in der Studienpopulation (Anteil am natürlichen indirekten Effekt in Relation zum Gesamteffekt: 28 % bei Männern; 33 % bei Frauen) und hochsensitiven CRPs (Prozentsatz von NIE des TE: 35 % bei Männern; 52 % bei Frauen). Dazu im Gegensatz war die WHR der schwächste Mediator. Allgemeine Adipositas, repräsentiert durch den BMI, vermittelte ungefähr ein Drittel der Assoziation von Bildung mit chronischer Entzündung bei älteren Menschen (Medenwald *et al.* 2015) in dem Studienkollektiv. Darüber hinaus war der Bildungsgrad mit echokardiografischen Parametern der linksventrikulären Masse assoziiert (Medenwald *et al.* 2016b).

Wurden die Inflammationsparameter als Exposition in die Analysen eingeschlossen, so zeigte sich, dass es vor allem der Rezeptor des TNF-alpha war, der eine Assoziation mit

verschiedenen kardiovaskulären Endpunkten zeigte: In der Querschnittsanalyse fanden sich Hinweise auf eine relevante Beziehung zwischen dem sTNF-R1 (Medenwald *et al.* 2014a) und verschiedenen echokardiografischen Parametern. Dieser Effekt war bei männlichen Versuchspersonen auf den Diameter des linken Vorhofes beschränkt. Im Gegensatz fanden sich bei Frauen nach Adjustierung für eventuelle Confounder ausgedehntere Relationen des sTNF-R1 mit verschiedenen echokardiografischen Parametern der kardialen Struktur (Hinterwand- und Septumdicke, linksventrikuläre Masse).

Wiederum war es der sTNF-R1, der in der Querschnittsanalyse mit der Länge der QT-Zeit assoziiert war. Nach Adjustierung für Kovariaten fand sich eine Odds Ratio (OR) von 1,89 (95-%-KI: 1,13, 3,17) pro 1000 pg/ml Anstieg des sTNF-R1 bei Frauen und 0,74 (95-%KI: 0,48, 1,15) bei Männern. In der Kovariaten-adjustierten linearen Regression war sTNF-R1 wiederum positiv mit der QT-Zeit bei Frauen assoziiert (5,75 ms pro 1000 pg/ml, 95-%-KI: 1,32, 10,18), nicht jedoch bei Männern. Unter Berücksichtigung möglicher Störfaktoren waren IL-6 und hsCRP bei beiden Geschlechtern nicht signifikant mit einer verlängerten QT-Zeit assoziiert (Medenwald *et al.* 2014c).

Die für die Herzfrequenz angepasste QT-Zeit war wiederum ein starker Prädiktor für die kardiovaskuläre Mortalität. Durch Hinzunahme der linksventrikulären Herzmasse wurde dieser Effekt im Sinne einer Interaktion modifiziert (Medenwald *et al.* 2016a). In Modellen, die wiederum für mögliche Confounder adjustiert wurden, war die QTc mit der allgemeinen Mortalität assoziiert, wobei das Hazard Ratio auf 1,19 mit 95-%-Konfidenzintervall von 1,03 bis 1,38 geschätzt wurde. Im Vergleich zu den obersten Tertilen zeigten Probanden mit dem geringsten linksventrikulären Masseindex (HR = 1,73, 95-%-KI: 1,26 bis 2,36) die stärkste Assoziation mit der allgemeinen Sterblichkeit, was auch für das unterste Tertil der diastolischen linksventrikulären Hinterwanddicke beobachtet wurde (HR = 1,49, 95-%-KI: 1,10 bis 2,02).

5 Diskussion

Zur Analyse möglicher Determinanten des Levels der Inflammation wurde gezeigt, dass der kausale Pfad mit dem Bildungsniveau als Exposition und anthropometrischen Größen als Mediatoren zu circa 30 bis 50 % den Gesamteffekt des Bildungsniveaus bestimmt.

Übergewicht ist in einer Reihe von Studien als ein Risikofaktor für die koronare Herzkrankheit auch in metabolisch gesunden Personen identifiziert worden, wie Zheng et al. in einem kürzlich publiziertem Review gezeigt haben (Zheng *et al.* 2016). Damit ist die

zentrale Rolle des Übergewichts als eines unabhängigen Risikofaktors einmal mehr bestätigt. Dieser Zusammenhang wurde auch in dem Kollektiv der CARLA-Studie gesehen. Dabei wurde besonders das erhöhte Risiko für Manifestationen der koronaren Herzkrankheit durch eine zentrale Adipositas betont (Pischon *et al.* 2008; Ekelund *et al.* 2015; Sharma *et al.* 2016).

Zur Fragestellung, inwieweit das Bildungsniveaus das Bevölkerungsmittel anthropometrischer Größen beeinflusst, die selbst Kenngrößen der Adipositas sind, wurde eine Reihe von Arbeiten publiziert, die ein erhöhtes Risiko für Übergewicht bei Personen mit geringem Bildungsniveau zeigten (Molarius *et al.* 2000; Gutiérrez-Fisac *et al.* 2002; Wardle *et al.* 2002). Grundsätzlich ist die Exposition des Bildungsgrades von dem sozioökonomischen Status zu trennen, wobei beide Begriffe häufig synonym verwendet werden und stark miteinander korrelieren (Grittner *et al.* 2006). Ein entscheidendes Problem besteht darin, den sozioökonomischen Status zu definieren und zu messen. Dies motivierte den Verfasser vorliegender Arbeit wesentlich, sich auf die Bildung als Exposition zu konzentrieren.

In einer umfangreichen gepoolten Analyse aus sieben Kohorten, zu denen auch die Daten der CARLA-Studie zählten, fanden Herzog et al. (Herzog *et al.* 2016) einen stärkeren Zuwachs des Gewichts und des Hüftumfangs bei Personen mit geringem Bildungsniveau um 0,1 % bei beiden Geschlechtern (95-%-KI für Männer: 0,06–0,20; und für Frauen: 0,06–0,12; Hüftumfang: 95-%-KI bei Männern: 0,01–0,45; und bei Frauen: 0,05–0,22) im Vergleich zu Probanden mit einem höheren Bildungsgrad. War das Einkommen die Exposition, so wurden für Frauen ähnliche Effekte beobachtet. Bei Männern konnte keine Gewichtsänderung in Abhängigkeit vom Einkommen nachgewiesen werden. Dies untermauert die Annahme, dass bei Fragestellungen der gesundheitlichen Prävention das Bildungsniveau der entscheidende Parameter ist, der das Gesundheitsverhalten determiniert.

Auf der Suche nach der möglichen Ursache für diese Effekte zeigte sich in einer weiteren Arbeit, basierend auf der CARLA-Studie (Tiller *et al.* 2015), eine deutlich geringere Gesundheitskompetenz bei Personen mit dem niedrigsten Bildungsniveau (Männer: −7,36; 95-%-KI; −11,44 bis −3,29, Frauen: –3,83; 95-%-KI: −6,50 bis −1,17). Dieser Unterschied war deutlich geringer, wenn das Einkommen betrachtet wurde (Männer: 1,68; 95-%-KI: −3,15 bis −0,21; Frauen: −1,28; 95-%-KI: −2,96 bis 0,39). Des Weiteren war die Gesundheitskompetenz stark linear negativ mit dem inzidenten Diabetes mellitus, dem Myokardinfarkt und dem Schlaganfall assoziiert. Unter Berücksichtigung dieser Daten kann der Gesundheitskompetenz und dem Gesundheitsverhalten bei der Entwicklung von Über-

gewicht eine Schlüsselrolle zugesprochen werden. In einer großen bevölkerungsbezogenen dänischen Studie konnte gezeigt werden, dass die Gesundheitskompetenz ein Mediator der Beziehung zwischen Bildungsgrad und Gesundheitsverhalten ist (Friis *et al.* 2016).

Der physiologische Ursprung der chronischen Entzündung liegt zu einem Großteil im viszeralen Fettgewebe (Pou *et al.* 2007; Lapice *et al.* 2009; Rutten *et al.* 2010). In den Ergebnissen der Daten aus der CARLA-Studie schein es aber gerade das allgemeine, ubiquitäre Übergewicht (repräsentiert durch den BMI) zu sein, das eine erhöhte Entzündungslast bestimmt. Jedoch wurde die Validität des WHR zur Quantifizierung des viszeralen Fettgewebes in einer weiteren Arbeit kritisiert (Melmer *et al.* 2013). In dieser Untersuchung zeigte der BMI die stärkste Korrelation (im Vergleich zu WHR oder dem Verhältnis aus Hüftumfang zu Körpergröße) mit laborchemischen Parametern wie dem Adiponectin, Insulin oder der Nüchternglukose. In einem weiteren Kollektiv aus jungen Erwachsenen zeigte die totale Adipositas ebenfalls eine starke Korrelation mit Inflammationsparametern wie dem CRP (Wärnberg *et al.* 2006). In einer Bevölkerungskohorte der KORA-Studie fanden die Autoren ähnliche Relationen im Zusammenhang des CRP (Thorand *et al.* 2006). Allerdings konnte die stärkste Korrelation der Gesamtfettmasse mit dem CRP nach Adjustierung für Faktoren des Lebensstils nur bei Frauen gesehen werden. Hier erklärte die Gesamtfettmasse 18,2 % der Streuung des CRP zwischen den Teilnehmern der Studie. Schlussfolgernd ist der kausale Pfad eines Unterschiedes der chronischen Inflammation in Relation zum Bildungsniveau mit dem Körperfett als Mediator in Einklang mit anderen Arbeiten zu bringen, die sich auf die Allgemeinbevölkerung als Studienkollektiv konzentrierten. Die Wirkungen dieser chronischen Inflammationslast sind nun Gegenstand des folgenden Teils der Diskussion, wobei hier echokardiografische und elektrokardiografische Parameter der linksventrikulären Hypertrophie im Fokus stehen.

Zu dieser Fragestellung fanden sich in der CARLA-Studie Hinweise auf eine Assoziation des sTNF-R1 mit der linksventrikulären Masse in der Querschnittsuntersuchung, wobei diese Beziehung nicht in der prospektiven Untersuchung gesehen wurde. Da Probanden mit kardialen Vorerkrankungen ausgeschlossen wurden, liegt der Gedanke nahe, dass diese schwache Assoziation am ehesten durch eine endogene chronische Entzündungsreaktion getrieben wird, die selbst unabhängig von der kardialen Leistungsfähigkeit ist.

In einer ähnlichen Querschnittsstudie (Takei *et al.* 2009) war der sTNF-R1 mit der linksventrikulären Masse nach Adjustierung für mögliche Confounder (demografische Kovariaten und mögliche Risikofaktoren) assoziiert. Probanden, deren sTNF-R1-Wert im untersten Quantil lag, hatten eine erhöhte Chance, eine linksventrikuläre Hypertrophie

zu haben (Odds Ratio: 1,84, 95-%-KI: 0,97–3,64). Auch nach Einschluss aller betrachteten Inflammationsparameter (sTNF-R1, IL-6, CRP) war allein der sTNF-R1 signifikant mit einer LVH assoziiert. In einer weiteren Arbeit der Forscher Roselló-Lleti et al. (Roselló-Lletí *et al.* 2012) wurde der sTNF-R1 wiederum als derjenige Parameter beschrieben, der im Vergleich zu TNF, IL-6 oder Interleukin-1RA die stärkste Assoziation mit einer prävalenten LVH zeigte. Analysiert wurde hier ein Kollektiv von asymptomatischen Patienten mit arterieller Hypertonie.

Die fehlende Assoziation der Inflammationsparameter mit der Änderung der linksventrikulären Masse kann eventuell durch den zeitlichen Zusammenhang zwischen laborchemischer Manifestation des sTNF-R1 und der Reaktion in Zielstrukturen erklärt werden. So zeigten Ueland et al. (Ueland *et al.* 2005), dass die Plasmakonzentration nach einem Myokardinfarkt bereits nach einem Monat wieder deutlich abfiel. In der gleichen Studie traten in der Gruppe mit einer erhöhten Plasmakonzentration des sTNF-R1 vermehrt Todesfälle innerhalb eines Zeitraums von einem Monat nach Krankenhausaufnahme auf. Diese naheliegende Verletzung einer Proportionalitätsannahme im Vergleich zur Gruppe mit einer geringen Plasmakonzentration des sTNF-R1 lässt eine akute Zielwirkung des Rezeptors vermuten. Jedenfalls ist dieser Zeithorizont deutlich kürzer als das vierjährige Follow-up in der CARLA-Studie.

Klinisch erscheint die Ejektionsfraktion als eine der relevantesten echokardiografischen Parameter. Hier konnte keine Assoziation mit den betrachteten Inflammationsparametern gezeigt werden. Dieser funktionelle Parameter ist durch eine hohe Interrate-Variabilität charakterisiert (Dittoe *et al.* 2007), was seine Nutzung in komplexen Studien einschränkt. Daraus resultieren eine hohe zusätzliche Variabilität dieser Zielgröße und letztlich eine Abnahme der Genauigkeit der Effektschätzung und damit der statistischen Power. Weiterhin liegt die Begründung nahe, dass strukturelle Änderungen möglicherweise zu einem gewissen Grad funktionell kompensiert werden können.

Bo et al. argumentierten (Bo *et al.* 2012), die ventrikuläre Hypertrophie sei ein Zustand chronischer Entzündung auf einem niedrigen Niveau. Dies ist insofern interessant, als es die fehlende prospektive Assoziation der Inflammationsparameter mit einer Änderung der kardialen Struktur erklären kann. Damit wäre die Änderung des Entzündungsniveaus abhängig von der ventrikulären Masse. Eine solche Beziehung lässt sich allerdings nur anhand einer Erhebung der Inflammation zum Zeitpunkt der Basisuntersuchung und des Follow-up schlüssig mit ausreichender Evidenz untersuchen. Dies ist Aufgabe zukünftiger Studien, die sich auf die CARLA-Studie stützen, da Werte zum Zeitpunkt des ersten Follow-up demnächst vorliegen werden.

Die Geschlechtsunterschiede, wie sie im Kollektiv der CARLA-Studie gesehen wurden, unterscheiden sich von den Ergebnissen der Forscher Iwashima et al. (Iwashima *et al.* 2007), die ähnliche Effektstärken bei Männern und Frauen beobachteten. Eine mögliche Erklärung liegt wiederum in unterschiedlichen Studienkollektiven (Iwashima *et al.* 2007), die sich bei Iwashima et al. aus Patienten mit arterieller Hypertonie rekrutierten. Weiterhin wurde in dieser Studie allein das CRP als Exposition in die Analyse eingeschlossen.

In enger Beziehung zur linksventrikulären Masse steht die QT-Zeit, die als zweiter klinischer Parameter auf seine Assoziation mit Inflammationsparametern untersucht wurde. Auch hier zeigte sich eine Assoziation des sTNF-R1 mit der Länge des QT-Intervalls. Ebenfalls konnten Geschlechtsunterschiede beobachtet werden (Medenwald *et al.* 2014c).

Die hohe Prävalenz (11,6 %) einer verlängerten QT-Zeit in unserem Kollektiv kann möglicherweise mit der ebenfalls hohen Prävalenz der arteriellen Hypertonie begründet werden, wie eine vorherige Arbeit entdeckte (Tiller *et al.* 2013). Johnson et al. (Johnson *et al.* 2011) schätzen die Prävalenz einer verlängerten QT-Zeit (Cut-off der QTc: 480 ms) in einem Patientenkollektiv mit Hypertonie auf 13 %, was in einer der CARLA-Studie ähnlichen Größenordnung liegt.

Das QT-Intervall hat seine elektrophysiologische Grundlage im Aktionspotenzial myokardialer Zellen. Unter der Annahme, die Plasmakonzentration des sTNF-R1 spiegele die Aktivität des TNF-alpha wider, lässt sich eine Veränderung des Aktionspotenzials in Relation zum TNF-alpha vermuten (Kleinbongard *et al.* 2011). Tierstudien geben Hinweise darauf, dass TNF-alpha die Expression von Kaliumkanälen regulieren kann (Petkova-Kirova *et al.* 2006). Würde die Dichte auswärts gerichteter Kaliumkanäle TNF-alpha-induziert reduziert werden, würde dies final in eine Verlängerung des Aktionspotenzials münden, die sich elektrokardiografisch in einer Verlängerung der QT-Zeit niederschlagen würde. Die genotypische Expression dieser auswärts gerichteten Kaliumkanäle ist geschlechtsabhängig, was auch eine unterschiedliche Anfälligkeit dieser Kaliumkanäle für die Wirkung von Entzündungsmediatoren in Männer und Frauen implizieren könnte (Gaborit *et al.* 2010). Ein Effekt des TNF-alpha wurde auch bei Calciumkanälen beobachtet, was aber auch das QT-Intervall verkürzen würde (Stengl *et al.* 2010). Duncan et al. (Duncan *et al.* 2010) berichteten von einer erhöhten Durchlässigkeit kardialer Calciumkanäle, die arrhythmogene Ereignisse hervorrufen kann. Zusammenfassend scheinen myokardiale Kaliumkanäle die entscheidende Zielstruktur des TNF-alpha zu sein, die die beobachtete verlängerte QT-Zeit abhängig vom Niveau des sTNF-R1 bewirkt.

Bei der Untersuchung der der Geschlechtsunterschiede in der Assoziation des sTNF-R1 mit der QT-Zeit war ein Entzug des Östrogens im Tiermodell mit einem Anstieg des TNF-alpha verbunden (Park *et al.* 2006). Zu dieser Argumentation passt auch ein Anstieg der Effektschätzer, nachdem prämenopausale Probandinnen und Frauen mit regelmäßiger Östrogeneinnahme ausgeschlossen worden waren. Allerdings ist das Kollektiv der CARLA-Studie aufgrund seiner Altersstruktur nicht geeignet, eine solche Fragestellung zu beantworten.

Die elektrophysiologische Rolle des IL-6 und CRP bleibt in dem betrachteten Studienkollektiv der CARLA-Studie unklar. Dieser Umstand wird dadurch erschwert, dass beide Variablen eine schiefe Verteilung aufweisen (siehe oben). Die Studienlandschaft für das CRP ist dünn. In einer Arbeit wurden nur geringe Effektschätzer für das CRP berichtet (Kim *et al.* 2006).

Werden nun die prädiktive Bedeutung echokardiografischer und elektrokardiografischer Parameter, vertreten durch das QT-Intervall, zusammengeführt, so lässt sich eine Interaktion zwischen beiden Größe in der CARLA-Studie in Relation zu kardiovaskulärer und allgemeiner Mortalität beobachten. Beide Parameter zeigen eine Assoziation mit dem sTNF-R1. Neben der Abschätzung einer möglichen Prädiktion des Überlebens durch diese Parameter bzw. Parametergruppen soll am Ende der Diskussion auf die Assoziation des sTNF-R1 mit dem Endpunkt des Überlebens eingegangen werden.

Die Bedeutung echokardiografischer Parameter insbesondere der Ejektionsfraktion im außerklinischen Setting bleibt anhand der hier genutzten Daten unklar. In der CARLA-Studie war die linksventrikuläre Masse nur ein Modifikator der Assoziation der QTc mit kardiovaskulärer Mortalität, wobei es keine Evidenz für eine eigene prädiktive Bedeutung der linksventrikulären Masse anhand dieser Daten gab. In einer finnischen Studie war die linksventrikuläre Masse nach Kategorisierung anhand eines Schwellenwerts von 120 g/m^2 mit Todesfällen durch plötzlichen Herztod assoziiert (Laukkanen *et al.* 2014). Aufgrund der geringen Fallzahl von 63 Fällen mit „Sudden cardiac Death“ ist das Konfidenzintervall bei einer Hazard Ratio von 2,57 weit (95-%-KI 1,24 bis 5,31), was eine deutliche statistische Unsicherheit andeutet. Die Autoren berechneten ebenfalls den Integrated Discrimination Index (IDI) zur Abschätzung der Diskriminationsfähigkeit (Todesfälle durch plötzlichen Herztod) des Modells gegenüber konventionellen Risikofaktoren. Im konkreten Beispiel verbesserte dies den IDI, indem der linksventrikuläre Massenindex hinzugezogen wurde, um 0,033 (95-%-KI 0,009 bis 0,057; P = 0,007). Einschränkend muss zu dieser Studie gesagt werden, dass diese allein 905 Männer mittleren Alters (42 bis 61 Jahre) betrachtete. Im Gegensatz zur CARLA-Studie war hier bereits ein Follow-

up nach 20 Jahren verfügbar. In einem Patientenkollektiv mit stabiler koronarer Herzkrankheit war eine erhöhte linksventrikuläre Masse ebenfalls mit dem Auftreten des „Sudden cardiac Death“ assoziiert (Turakhia *et al.* 2008). Der deutliche Unterschied zu einem Setting in der Allgemeinbevölkerung zeigt sich schon in einer merklich geringeren Beobachtungszeit, die in der Studie der Forscher Turakhia et al. nur 3,6 Jahre betrug. In der Arbeit von Laukkanen et al. (Laukkanen *et al.* 2014) wurde diese Variable der linksventrikulären Masse dichotomisiert, was mit einem deutlichen Informationsverlust einhergeht. Grundsätzlich wäre eine lineare Beziehung zwischen Exposition und Outcome im Sinne einer Kausalität notwendig, wenn ein größerer Wert des Outcomes bei stärkerer Exposition angenommen wird.

Zur Frage nach der Beziehung zwischen der linksventrikulären Masse und der QT-Zeit untersuchten Mukerji et al. (Mukerji *et al.* 2012) ein Kollektiv von Übergewichtigen als Teil einer prospektiven Kohortenstudie. Die Autoren fanden eine starke Beziehung zwischen der linksventrikulären Masse und der für die Herzfrequenz korrigierten QT-Zeit ($r = 0{,}793$). Diese starke Korrelation beider Größen ist besonders in diesem Kollektiv bemerkenswert, da die verlässliche Aufzeichnung des EKGs und die korrekte Durchführung der echokardiografischen Untersuchung bei übergewichtigen Probanden zu Messfehlern führen können, die selbst wiederum in eine größere Varianz der Daten münden.

Einer ähnlichen Fragestellung gingen Chapman et al. (Chapman *et al.* 2001) in einem Kollektiv aus 386 nicht selektionierten Probanden mit nachgewiesener Hypertension nach. Hier zeigte die maximale gemessen QT-Zeit eine starke Korrelation mit der linksventrikulären Masse, wenngleich die Korrelation etwas schwächer geschätzt wurde als bei Mukerji et al. ($r = 0{,}25$). Oikarinen et al. (Oikarinen *et al.* 2003) fanden im Rahmen der „Losartan Intervention For Endpoint Reduction Study“ (LIFE-Studie) ebenfalls eine Assoziation zwischen der QT-Zeit und der linksventrikulären Masse im Sinne einer positiven Assoziation.

In der Kohorte der CARLA-Studie zeigten beide Parameter eine Assoziation mit dem sTNF-R1, wenn eine Querschnittsuntersuchung durchgeführt wurde. Zu dieser Relation sind nur wenige Studien in der Literatur verfügbar. Das zirkulierende TNF-alpha, nicht sein löslicher Rezeptor, korrelierte in einer kleinen Kohorte von Patienten mit einer rheumatischen Erkrankung mit der QT-Zeit (Adlan *et al.* 2015). Nach Adjustierung für das Patientenalter verschwand diese signifikante Relation.

Eine Arbeitsgruppe um Cox et al. (Cox *et al.* 2014) befasste sich mit dem Mortalitätsrisiko bei verlängerter QT-Zeit in Patienten mit prävalentem Diabetes mellitus, Typ 2. Es zeigte sich eine Assoziation der QT-Zeit mit der Gesamtmortalität. Für einen Anstieg der QT-

Zeit um eine Standardabweichung wurde eine Risikoerhöhung von 18 % geschätzt (Hazard Ratio 1,18; 95-%-KI: 1,03–1,36). Die enge Beziehung zwischen der QT-Zeit und der linksventrikulären Hypertrophie wird auch durch Daten aus der CARLA-Studie verdeutlicht (Medenwald *et al.* 2016a). Hier fand sich eine deutliche Assoziation der QT-Zeit mit der kardiovaskulären und Gesamtmortalität. Eine ähnliche Relation konnte für die linksventrikuläre Masse nicht gezeigt werden. Interessanterweise war die Vorhersagekraft der QT-Zeit für die Mortalität stark abhängig von der linksventrikulären Masse im Sinne einer Interaktion beider Parameter. Dieser kurze Review verdeutlicht die starke Beziehung zwischen beiden Größen.

Diese Ergebnisse bestätigen eine weitere Studie der Forscher Haugaa et al. (Haugaa *et al.* 2014), die in einem Krankenhauskollektiv eine Assoziation zwischen der QT-Zeit und dem Überleben zeigte (Hazard Ratio: 1,31 per 10 ms Zunahme der QTc; 95-%-KI: 1,09–1,58). Der Effekt war in derjenigen Gruppe am stärksten, die eine konkomitante, im EKG diagnostizierte, ventrikuläre Hypertrophie zeigte, wobei die Autoren den Cut-off für eine verlängerte QT-Zeit bei einer QTc von 500 ms definierten. Einschränkend muss zu dieser Arbeit gesagt werden, dass die QT-Zeit hier allein elektrokardiografisch bestimmt wurde. Diese Form der Bestimmung einer ventrikulären Hypertrophie wurde in zahlreichen Studien infrage gestellt. Der Cut-off von 500 ms ist kaum relevant für Kohorten der Allgemeinbevölkerung. So wurde eine solche extrem verlängerte QT-Zeit bei nur 13 Fällen gesehen, unter denen fünf Todesfälle beobachtet wurden. Die Interaktion zwischen QT-Zeit und linksventrikulärer Hypertrophie in Relation zum Überleben in der CARLA-Studie beruht maßgeblich auf echokardiografischen Parametern der kardialen Wanddicke (Porthan *et al.* 2007). Störungen der Repolarisation wurden gerade bei einer erhöhten Wanddicke beobachtet, was die Argumentation stützt, dass eine Verlängerung der QT-Zeit durch eine hypertrophieassoziierte Repolarisationsstörung den prädiktiven Wert der QT-Zeit abschwächt.

In enger Verbindung zu der Assoziation des QT-Intervalls mit der Mortalität war auch das CRP und IL-6 mit dem Überleben in dem Kollektiv der CARLA-Studie assoziiert. Die Einbeziehung des NT-proBNP reduzierte die Effektstärke deutlich. Dieser Effekt war besonders im Fall des möglichen prädiktiven Effekts des sTNF-R1 ausgeprägt. In analoger Weise zu dem QT-Intervall und der ventrikulären Masse wirkte die HRV als Effektmodifikator. Zwischen beiden Parametern kam es mithin zu einer Interaktion zwischen dem sTNF-R1 und HRV-Parametern des autonomen Nervensystems auf der additiven Skala. Wie eingangs erwähnt, ist diese Skala besonders für die Untersuchung biologischer Prozesse der multiplikativen Skala vorzuziehen (Rothman *et al.* 2008).

Singh und Newman (Singh & Newman 2011) nennen in ihrem Review zur Assoziation von Inflammationsparametern mit dem Überleben verschiedene Studien. Die schwachen Assoziationen im CARLA-Kollektiv wirken hier etwas überraschend. Doch muss dies vor dem Hintergrund betrachtet werden, dass hier eine Adjustierung für das NT-proBNP erfolgte, das seinerseits ein metrischer Parameter ist (und sich damit selbst als Exposition über ein weiteres Spektrum bzw. eine große Varianz erstreckt), der stark mit dem Überleben assoziiert ist.

Frühere Studien weisen darauf hin, dass eine Entzündung den vagalen Tonus erhöht, während eine erhöhte parasympathische Aktivität ein starker Inhibitor der TNF-alpha-Freisetzung im Sinne eines entzündungsregulierenden Feedbacks ist (Huston & Tracey 2011). Durch die negative Korrelation des sTNF-R1 mit der HRV des parasympathischen Nervensystems scheint diese Beziehung in der CARLA-Studie bestätigt. Dies ist ebenfalls durch den starken Effekt von HRV-Parametern, die dem parasympathischen Ast des autonomen Nervensystems zugeordnet werden (High Frequency Power), bestätigt (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology 1996).

Wie schon an anderer Stelle erwähnt, erlaubt es der Querschnittsansatz nicht, die kausale Richtung des Effekts zu determinieren. Aus experimentellen Studien ist bekannt, dass es auch zu einer entzündungsbedingten Abnahme der HRV und damit einer Änderung der Aktivität des autonomen Nervensystems kommen kann (Hajiasgharzadeh *et al.* 2011).

In ähnlicher Form ließe sich die gefundene Interaktion zwischen Entzündungsparametern, der HRV und damit des autonomen Nervensystems begründen. Ein reduzierter parasympathischer Tonus ist demnach selbst mit einem erhöhten Mortalitätsrisiko assoziiert. Gleichzeitig könnte dieser verminderte Tonus zu einer ebenso reduzierten Kapazität führen, um auf eine erhöhte Entzündungslast mit einer entsprechenden Anpassung des Regelkreises zu reagieren. Sajadieh et al. (Sajadieh *et al.* 2006) fanden eine Interaktion des CRP mit HRV-Parametern, was in dem Kollektiv der CARLA-Studie nicht zu beobachten war. In der genannten Studie wurden Probanden mit kardialen Erkrankungen ausgeschlossen, was zu einem Kollektiv führt, das sich von der CARLA-Kohorte mit einer starken Prävalenz der Hypertonie merklich unterscheidet.

5.2 Limitationen

Für die Analyse des Konzepts des sozioökonomischen Status wurde dieser hier nur eindimensional mittels des Bildungsniveaus definiert. Dieses Vorgehen hat Vorteile aufgrund seiner einfachen Definition und größeren Bedeutung gegenüber dem Einkommen, wie in der Diskussion besprochen (Grittner *et al.* 2006; Tiller *et al.* 2015; Herzog *et al.* 2016). Dennoch kann eine Analyse eines mehrdimensionalen Konzepts, sprich des sozioökonomischen Status, weitere Beziehungen aufzeigen, die nicht allein vom Bildungsniveau abhängen.

Um die Frage nach einer kausalen Beziehung zu beantworten, wäre es notwendig, eine zeitliche Veränderung chronischer Inflammationsparameter in Abhängigkeit von Parametern zum Zeitpunkt der Basisuntersuchung zu analysieren. Jedoch wurden in der CARLA-Studie der sTNF-R1 und das IL-6 nur einmalig zum Basiszeitpunkt gemessen. Es kann somit keine Aussage zur Abhängigkeit einer Veränderung der Blutkonzentration beider Entzündungsparameter von Größen der Baseline-Untersuchung getroffen werden. Dies wäre jedoch ein essenzieller Bestandteil für Kausalität im Kontext beobachtender Studien, wie sie Sir Austen Bradford Hill eingeführt hat. Im Detail proklamierte er die Bedingung der Zeitlichkeit (*Temporality*) i. S. einer zeitlichen Nachrangigkeit des Effekts gegenüber dem Effektor (Hill 2015).

Für die Messung echokardiografischer Parameter stellt die hier genutzte Methode nach Teichholtz sowohl im klinischen als auch im wissenschaftlichen Setting keinen Standard mehr dar. Mit der Implementierung des Gewebedopplers und dreidimensionaler Verfahren können nun die kardiale Funktion und Struktur genauer und verlässlicher bestimmt werden (Lang *et al.* 2015). Aus einer klinischen Perspektive erscheint das Outcome echokardiografischer Werte weniger relevant als die Einbeziehung entsprechender krankheitsassoziierter Symptome. Jedoch war die Fragestellung hier auf diese etwas technischere Zielgröße ausgerichtet. Die Fragestellung im Sinne einer Vorhersage der symptomatischen Herzinsuffizienz anzupassen, würde zu einer veränderten Analysemethode führen und durch ein subjektives Moment in der Symptomdefinition eingeschränkt sein. Die Bestimmung echokardiografischer Größen zeichnet sich durch eine unter Umständen bemerkenswerte Intra- und Interrater-Variabilität aus. In der CARLA-Studie variierte die mittlere Beobachterabweichung zwischen 0,3 % und 3,8 % (2 × SD zwischen 15,3 % und 27,7 %), während die Variabilität unter den Beobachtern zwischen 0,1 % und 2,7 % lag (2 × SD zwischen 12,7 % und 20,8 %). Auch diese Streuung der Zielgröße führt zu einer Zunahme der statistischen Unsicherheit bei der Berechnung entsprechender Effektschätzer.

Auf der Seite der inflammatorischen Parameter ist es gerade die chronische Entzündung, für die eine Assoziation mit kardiovaskulärer Mortalität angenommen wurde. Letztlich lässt sich eine solche subklinische Manifestation allein anhand wiederholter Messungen klar von einer akuten Entzündungsreaktion abgrenzen (Hardikar *et al.* 2014).

Grundsätzlich ist eine Beeinflussung der Elektrolyte durch Entzündungsreaktionen denkbar, was letztlich in eine Verlängerung der QT-Zeit münden kann. In der CARLA-Studie wurden allerdings keine Elektrolyte im Blut bestimmt, sodass kein direkter Effekt des TNF-alpha von einem indirekten Effekt mit Elektrolyten als Mediator differenziert werden kann.

Da das Studienkollektiv auf die Allgemeinbevölkerung ab einem Alter von 45 Jahren restringiert wurde, lassen sich keine Aussagen über das Langzeitrisiko jüngerer Personen treffen, wobei gerade in dieser Bevölkerungsgruppe die größten Potenziale einer Prävention kardialer Erkrankungen liegen. Mit dieser Argumentation eng verknüpft ist die Vorstellung eines weniger stark ausgeprägten Confounding durch entzündungsinduzierende Komorbiditäten in jüngeren Zielgruppen.

Die hier als Kovariaten betrachteten Parameter beruhten zum großen Teil auf der Selbstauskunft der Probanden. Zwar ist ein solcher Ansatz einfach in eine interviewbasierte Datenerhebung zu implementieren, doch ist er auch anfällig für differenzielle und nicht differenzielle Fehlklassifikationen (Rothman *et al.* 2008). Im Falle des Diabetes mellitus lässt sich dieser mögliche Bias noch zu einem gewissen Grade durch die zusätzliche Einbeziehung des HbA1c relativieren, was jedoch bei einer Variablen wie dem anamnestischen Myokardinfarkt sehr viel schwieriger ist. Eine ärztliche Evaluation der Selbstangaben wurde jedoch bisher nicht im Rahmen der CARLA-Studie realisiert.

In enger Beziehung dazu steht die Möglichkeit einer unvollständigen Adjustierung für mögliche Confounder des Effekts der anthropometrischen Parameter mit der Inflammation. Dieser Umstand erscheint besonders dramatisch bei Fragestellungen im Zuge von Mediatoranalyen. Valeri und VanderWeele (Valeri & Vanderweele 2013) zeigen Möglichkeiten auf, eine unvollständige Adjustierung für mögliche Confounder abzuschätzen. Diese Verfahren wurden punktuell im Falle von Mediatoranalysen angewandt. Zusammenfassend kann gesagt werden, dass eine grundsätzliche Änderung der gefundenen Beobachtungen im Sinne einer Verschiebung der Konfidenzintervalle in einen Bereich, der den Nulleffekt einschließt, unwahrscheinlich erscheint. Allerdings können diese Verfahren nur bedingte Aussagen zur der geschätzten Effektgröße selbst treffen, was damit eine quantitative Verschiebung der Effektschätzer durch nicht betrachtete Confounder

möglich erscheinen lässt (Valeri & VanderWeele 2013). Wie eingangs erwähnt, stellten Valeri und VanderWeele (Valeri & VanderWeele 2013) vier Bedingungen auf, um kausale Rückschlüsse ziehen zu können, von denen eine vollständige Adjustierung ein Teil ist. Es ist grundsätzlich zu diskutieren, inwieweit diese Bedingungen bei den Möglichkeiten der CARLA-Studie erfüllt sind. Diese mögliche Nichterfüllung der notwendigen Bedingungen nach Valeri und VanderWeele ist am bedeutsamsten, wenn eine unvollständige Adjustierung für mögliche Confounder vorliegt. Im Detail sind dies mögliche Confounder der Beziehung zwischen Bildungsniveau und Übergewicht sowie der Relation zwischen anthropometrischen Parametern und der Inflammation, wie oben bereits erwähnt. Dass das Bildungsniveau selbst ein Confounder der Assoziation zwischen den genannten anthropometrischen Parametern ist, erscheint unwahrscheinlich, da es kaum zu begründen ist, warum sich die Physiologie des Fettgewebes in Abhängigkeit vom Bildungsniveau unterscheiden soll.

Wie bei der Mehrzahl der in einer einzelnen Studie beobachteten Effekte müssen diese Ergebnisse in anderen unabhängigen Kohorten reproduziert werden, um Effekte zu vermeiden, die sich durch eine Überanpassung ergeben könnten (Mallett *et al.* 2010). Allerdings wurde im Rahmen dieser Arbeit nur die CARLA-Kohorte betrachtet. Eine Lösung zu diesem Problem würde darin liegen, die Studienpopulation in eine Trainings- und eine Test-Kohorte zu teilen. Wegen des begrenzten Stichprobenumfangs ist es nicht möglich, dieses Verfahren effizient anzuwenden, da die statistische Power deutlich reduziert werden würde.

Die Bestimmung eines einzelnen Markers erlaubt es nicht, auf komplexe Reaktionen der Immunantwort zu schließen. Blutparameter wie der sTNF-R1 sollten daher allein als Biomarker interpretiert werden, deren klinischer Wert sich an der Verbesserung bestehender Risikoprädiktionsmodelle wie des Framingham Risk Score (Wilson *et al.* 1998) messen muss. Ein solches Vorgehen wurde durch Tzoulaki et al. anhand eines systematischen Reviews demonstriert (Tzoulaki *et al.* 2009). Die Autoren identifizierten dabei 79 Arbeiten, die eine Verbesserung des Framingham Risk Score durch Hinzunahme eines weiteren Faktors analysierten. Unter den eingeschlossenen Studien waren allerdings 49, die den Framingham Risk Score nicht korrekt berechneten.

6 Ausblick und Zusammenfassung

In dieser Arbeit wurde unter dem Aspekt des klinischen Outcome primär die kardiovaskuläre bzw. Gesamtmortalität betrachtet. Trotz seiner vielen Vorteile wie zum Beispiel der Möglichkeit, semi-parametrische Verfahren wie die Cox-Regression zur Analyse zu nutzen, der klaren Definition des Endpunktes bzw. zensierter Ereignisse könnte gerade die Analyse krankheitsbezogener Endpunkte wie des akuten Myokardinfarkts weitere Einsichten in die Bedeutung von Inflammationsparametern schenken. Grundsätzlich ist die chronische subklinische Endzündungsreaktion ein ubiquitärer Prozess, der eine zentrale Bedeutung über den Bereich der Kardiologie hinaus zeigt. Verschiedene Studien zeigten die Bedeutung immunologischer Prozesse in der Kanzerogenese (Elinav *et al.* 2013; Carlsson *et al.* 2014; Diakos *et al.* 2014). Letztlich zeigen bevölkerungsbezogene Analysen die gleichen Vorteile in der Untersuchung der zweiten großen Gruppe

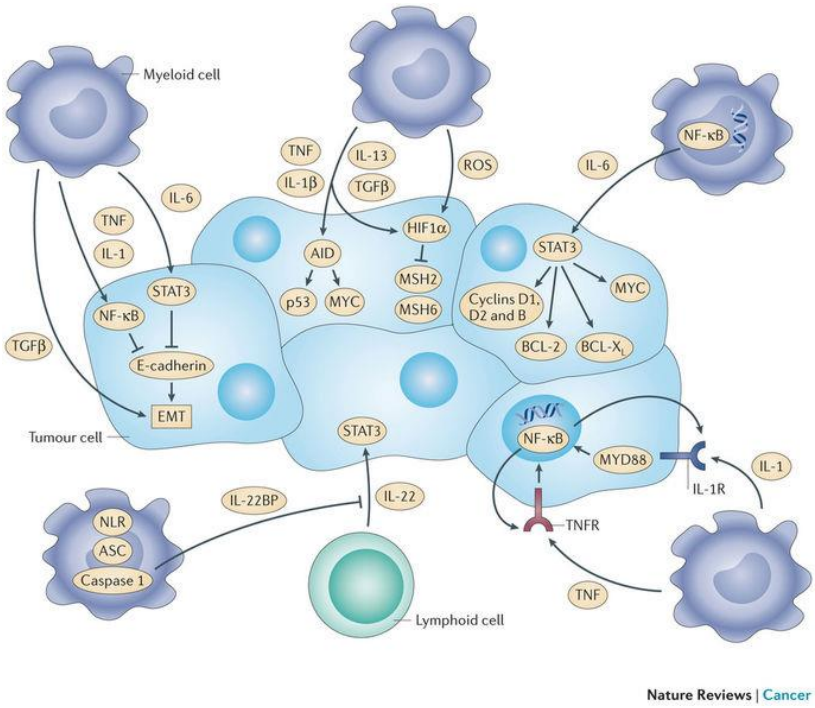

Abbildung 5: Relation von Entzündungsprozessen im Rahmen der Kanzerogenese nach Elinav et al. 2013

Pro-inflammatorische Zytokine stoßen verschiedene Signalwege und Aktivatoren der Transkription wie das STAT3 und den nuklearen Faktor-κB (NF-κB) in Krebszellen an, was zur Unterdrückung der Apoptose führt.

Abkürzungen: ASC, Apoptosis-associated Speck-like Protein containing a CARD; HIF1α, Hypoxia-inducible Factor 1α; IL-1R, IL-1 Receptor; MYD88, Myeloid Differentiation primary Response 88; NLR, NOD-like Receptor; ROS, Reactive Oxygen Species; TGFβ, Transforming Growth Factor-β; TNF, Tumor Necrosis Factor; TNFR, TNF Receptor.

bedeutender Todesursachen, sprich des onkologischen Formenkreises, wie im kardiologischen Umfeld. Aufgrund der längeren Latenzzeit bis zur klinischen Manifestation der Erkrankung sind längere Follow-up-Zeiten erforderlich. Die hier untersuchten Inflammationsparameter wie das Interleukin-6 oder die löslichen TNF-Rezeptoren sind in ein breites Netzwerk der entzündungsbedingten Kanzerogenese eingebettet. In der Arbeit der Forscher Elinav et al. (Elinav *et al.* 2013) lässt sich dieser komplexe Mechanismus erahnen. In diesen Interaktionen liegen die möglichen Angriffspunkte für zukünftige Forschungsprojekte.

Bisher wurde der lösliche TNF-Rezeptor nur in wenigen Studien neben der Basisuntersuchung auch in einem Follow-up bestimmt (Carpena *et al.* 2012; Roselló-Lletí *et al.* 2012). In der Arbeit der Forscher Roselló-Lletí et al. (Roselló-Lletí *et al.* 2012) wurde ein rein klinisches Kollektiv aus 220 Patienten nach einem Zeitintervall von im Mittel zwei Jahren auf eine etwaige Änderung des NT-proBNP hin analysiert. Wenngleich die zeitliche Variation des sTNF-R1 nicht die primäre Zielgröße in dieser Studie darstellte, so zeigte sich doch eine starke Korrelation mit dem Level des NT-proBNP, was ein gleich gerichtetes Verhalten beider Parameter in einer prospektiven Erhebung nahelegt. Letztlich sind klinische Daten aber durch medizinische Interventionen anfällig für Verzerrungen.

Die Änderung der Blutkonzentration dieses Biomarkers könnte neben dem Messwert zum Basiszeitpunkt mit dem Überleben assoziiert sein. In diesem Zusammenhang wäre es auch möglich, die zeitliche Veränderung der löslichen Rezeptoren in Abhängigkeit von verschiedenen chronischen und akuten, mit einer erhöhten Entzündungsreaktion einhergehenden Krankheitszuständen zu schätzen.

Zusammenfassend zeigen diese verschiedenen Analysen, dass die chronische Entzündung einen weitreichenderen Effekt auf das kardiovaskuläre Organsystem und das Überleben zeigt. Besonders der lösliche Rezeptor des TNF-alpha erweist sich hier offenbar als ein neuer, vielversprechender Biomarker. Jedoch bleibt seine Rolle in einem weiteren epidemiologischen Kontext unklar. Dies betrifft an erster Stelle den Effekt der zeitlichen Variation und den möglichen Effekt einer Medikation bei prävalenten kardialen Erkrankungen. Ebenso kann dieser Rezeptor nur im Kontext weiterer Erkrankungen wie renaler Störungen betrachtet werden (Medenwald *et al.* 2014b). Dies verlangt nach weiteren Studien, die sich solcher Kollektive annehmen, die ein besonderes kardiovaskuläres Risiko aufweisen. Die Ergebnisse der CARLA-Studie können hierzu einen ersten Anstoß bilden.

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Anhang

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Educational level and chronic inflammation in the elderly – the role of obesity: results from the population-based CARLA study

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What is already known about this subject?

- Lower social classes are characterized by a higher load of chronic inflammation.
- Visceral and abdominal fat contribute to chronic inflammation, whereas obesity is more prevalent in lower social classes.
- In adolescence, parameters related to obesity were strong mediators of an association of education with chronic inflammation.

What does this study adds?

- Lifetime education is associated with chronic inflammation in the elderly general population.
- Roughly one-third of the effect of education on chronic inflammation can be explained by general obesity.
- In comparison with body weight, waist-to-hip ratio explains a smaller amount of the total effect of education on chronic inflammation.

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Summary

This study aimed to assess the mediating role of anthropometric parameters in the relation of education and inflammation in the elderly. Cross-sectional data from the population-based CARdio-vascular Disease, Living and Ageing in Halle study were used after excluding subjects with a plasma level of high-sensitive C-reactive protein (hsCRP) above 10 mg L^{-1} (916 men/760 women remaining). Education was categorized in accordance with International Standard Classification of Education. As inflammation parameters, the soluble tumour necrosis factor type 1 (sTNF-R1), hsCRP and interleukin 6 (IL-6) were taken into account. Anthropometric parameters were the body mass index (BMI), waist-to-hip ratio (WHR) and waist-to-height ratio (WHeR). We used covariate adjusted mixed models to assess associations. Effect measures were the natural indirect effect (NIE), controlled direct effect and total effect (TE). Education was associated with sTNF-R1, hsCRP and IL-6 in men, and sTNF-R1 and hsCRP in women. Anthropometric parameters correlated with all inflammation parameters after covariate adjustment. BMI and WHeR were strong mediators of educational differences in sTNF-R1 (percentage of NIE of TE: 28% in men; 33% in women) and hsCRP (percentage of NIE of TE: 35% in men; 52% in women), while WHR was the weakest mediator. General obesity mediates roughly one-third of the association of education with chronic inflammation in the elderly.

Keywords: Education, inflammation, mediation, obesity.

Introduction

The pathogenic and prognostic value of chronic, low-grade inflammation has been of major research interest. Probably, the most widely examined inflammation parameter is C-reactive protein (CRP), an acute phase protein that has been related to cardiac illnesses, such as congestive heart failure (1), and pathologic conditions, such as left

ventricular hypertrophy (2), arteriosclerosis (3) and electrophysiological changes (4).

Besides CRP, interleukin 6 (IL-6) is related to cardiovascular diseases and conditions, mostly because of its prognostic relevance in clinical collectives (5) of the elderly (6).

Apart from them, further cytokines are linked to cardiovascular morbidity and chronic inflammation. As a major inflammation component, the tumour necrosis factor alpha (TNF-alpha) system seems to be involved in several pathways leading to adverse alterations of tissues (7,8). Nevertheless, temporal fluctuations of TNF-alpha make it difficult to assess chronic inflammation by measuring blood levels of this parameter (9). The soluble TNF-alpha type 1 and 2 receptors (sTNF-R1/2) were found to be temporally stable markers of TNF-alpha activity (9), thus making them useful surrogate markers of chronic inflammation. The prognostic relevance of soluble TNF receptors in cardiovascular diseases was revealed by several previous studies (10). Even more, sTNF-R2 (11) was found to be a substantial predictor of a loss of renal function in a collective of the general population.

Explaining individual differences in inflammation levels, a low socio-economic status and education were associated with a higher blood level of CRP (12). IL-6 was also higher in low social economic classes and inversely associated with education (13). In a collective of the Framingham Heart Study, a higher socio-economic position was associated with a lower level of TNF-alpha receptor type 2 (14). In contrast to CRP and IL-6, the effect was robust after covariate adjustment.

By causally linking socio-economic status/education and inflammation, body weight might mediate the observed relations of social status and inflammation, as it is well established that fat tissue is an important source of chronic inflammation (15–17). Such a mediation effect of body mass index (BMI) was found in a collective of adolescents, explaining the majority of the association of social status on plasma inflammation parameters (18). However, the mediation effect of further anthropometric parameters such as waist-to-hip ratio (WHR) representing fat distribution remains insufficiently investigated. As it was hypothesized that visceral/abdominal fat is the main source of inflammation and increased cardiovascular risk (17,19) such investigations might reveal further insights in the underlying processes. Although inflammation is an important new risk factor for cardiovascular diseases and the TNF-alpha was found to be a key component in inflammation-induced cardiovascular risk (10), little research has been conducted as to the long-term effect of early socio-economic inequalities on plasma levels of this new cardiovascular biomarker. Even more, the plasma level of inflammation offers a unique way to assess cardiovascular risk more objectively as compared with self-reported information such as smoking status or diet.

Considering previous findings, our study aims to investigate (i) the association of parameters representing socio-economic status/education with parameters of chronic inflammation with a special focus on gender differences. Additionally, we aim to determine a possible mediation of this association by anthropometric parameters related to obesity (ii). Extending previous research, we intent to assess possible differences in a mediation effect by generally and abdominally distributed fat (iii).

Methods

Study cohort

We used data from the *CARdio-vascular Disease, Living and Ageing in Halle* study (CARLA study), which is a prospective population-based cohort study of the elderly general population of the city of Halle in Eastern Germany (20). The CARLA cohort comprises 1779 participants aged 45–83 years at baseline (812 women, 967 men). The baseline examination took place between December 2002 and January 2006. A multi-step recruitment strategy aimed to achieve a high response rate. The final response rate percentage after subtracting exclusions (individuals who were deceased prior to the invitation, had moved away or were unable to participate due to illness) was 64%. All data used in this cross-sectional analysis resulted from the baseline examination of the study. The study participants underwent a detailed medical examination and a standardized, computer-assisted interview, which collected information on socio-demographic and socio-economic variables; behavioural, biomedical and psychosocial factors; medical history; and the use of medication within the preceding 7 days. Medication was automatically coded according to the Anatomical Therapeutic Chemical Classification System (code). Additionally, an analysis of non-respondents was performed in order to assess non-response bias by obtaining information about prevalent diseases, and selected behavioural and socio-demographic factors. A more comprehensive account of the CARLA study can be found in Greiser *et al.* (20). The study was approved by the Ethics Committee of the Medical Faculty of the Martin-Luther-University Halle-Wittenberg and by the State Data Privacy Commissioner of Saxony-Anhalt and conformed to the principles outlined in the Declaration of Helsinki (21). All participants gave written informed consent.

As a CRP >10 mg L^{-1} indicates acute infections, subjects ($n = 103$) above this threshold were excluded from the analysis (22).

Laboratory measurements

Blood samples were taken after a supine rest of 30 min. The inflammation parameters of sTNF-R1 were analysed by the

Department of Medicine III, University Clinics Halle (Saale). After a 10-min centrifugation (20°C, 1500 rpm, Acc = 9, Dcc = 3), the plasma was collected and stored at –80°C. The cytokines were determined using commercially available sandwich enzyme-linked immunosorbent assays (ELISAs: IL-6, Opteia, BD Biosciences, Heidelberg, Germany; TNF-R1, Boehringer Mannheim, Mannheim, Germany).

The determination of CRP was undertaken by the Institute of Laboratory Medicine, Clinical Chemistry and Molecular Diagnostics at the Leipzig University Clinics. The laboratory has been accredited according to the accreditation norms ISO 15180 and ISO 17025. Serum levels of high-sensitive CRP (hsCRP) were measured using a high-sensitivity immunoturbidimetric method (CRP [Latex] HS, Roche Diagnostics, Mannheim, Germany) on a Hitachi autoanalyser (Roche Diagnostics).

Anthropometric parameters

For the analyses we took the following anthropometric parameters into account: the BMI, the WHR and the waist-to-height ratio (WHeR).

The anthropometric measurements followed the procedures used in the MONICA/KORA and SHIP study (23,24). Weight and height were measured with the SECA 701 (seca gmbh & co.kg, Halle, Germany) digital scale and the SECA 220 (seca gmbh & co.kg, Halle, Germany) height measuring system. Waist and hip circumference were measured using a flexible tape, with the study subject standing in front of a full-sized mirror, which allows checking the horizontal position of the tape. Weight was recorded with a precision of 100 g, and height, waist and hip circumference to the nearest 0.1 cm.

Educational level

As the self-reported information on income might be subjected to considerable bias, we focused solely on education as a parameter of socio-economic status. Educational level was defined in accordance with the International Standard Classification of Education version 1997 (25). Categories were formed as follows, with the indication of the years of education in brackets: low (9/10 years), medium low (11–13 years), medium high (14–17) and high education (18–20 years).

Further parameters

Information about rheumatic diseases, presence or history of cancer, smoking habit, health conviction and daily alcohol intake was inquired using a computer-based interview and subsequently reviewed by a physician.

Statistical methods

Descriptive results (Table 1) with their respective 95% confidence limits are displayed as arithmetic or geometric means in the case of skewed distributions. Mixed models were used to examine (i) the association of education (categorized and linear trend) with inflammation and, as separate analyses, (ii) the relations of education with anthropometric parameters and (iii) the association of anthropometric parameters with inflammation. Considering directed acyclic graphs (28), we adjusted models of the education–anthropometric parameter association (Fig. 1) for the parameters listed in Table 1 (column C1). The model examining the association of anthropometric parameters with sTNF-R1 was adjusted for the confounders grouped in the column C2 in Table 1. Both sets of confounders, C1 and C2 (C = [C1, C2]), were used for the mediation analysis (see 'Mediation analysis'). We used

Table 1 Covariates used in the analyses

	C1	C2
Body mass index	Age, gender, neighbourhood and household size	Age, gender, bronchitis, currently smoked cigarettes, cigars, pipes, sport index (26), daily alcohol intake, presence of rheumatoid arthritis or cancer, TSH, food index (27) and health conviction in terms of obesity

TSH, thyroid-stimulating hormone.

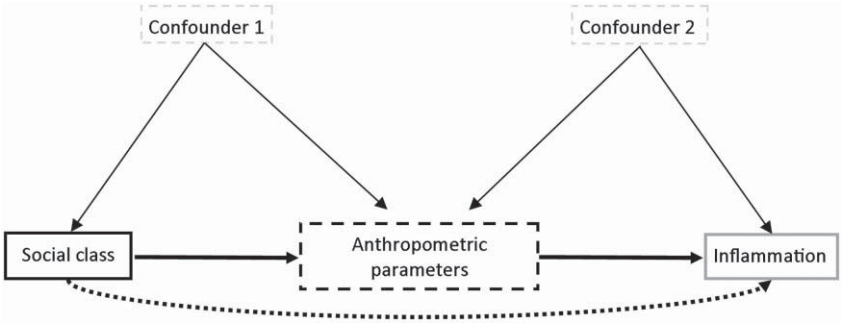

Figure 1 Directed acyclic graph of considered relations. Dashed arrow: controlled direct effect; undashed arrows: natural indirect effect.

mixed models with neighbourhood as a random factor and a first autoregressive covariance matrix (improper conditional autoregressive covariance matrices) to adjust for a potential confounding effect of neighbourhood (29); 39 separate neighbourhoods were considered in the statistical models. Unadjusted results are given in the supplement (Tables S2–S4). To make estimates comparable between analyses, parameters were standardized by dividing by the respective standard deviation. Inflammation parameters were log-transformed because of skewed distributions. After retransformation, positive/negative estimates refer to a percentage increase/decrease in the dependent parameter per unit increase of the independent parameter.

Mediation analysis

For the mediation analysis, we used the approach introduced by Valeri and Vanderweele (30). As there were no significant exposure–mediator interactions when an interaction term (one exposure*mediator interaction per model) was added to the regression model, we respected main effects only (no interaction term such as education*BMI included in the respective models used for mediator analyses, results not shown). As effect measures the controlled direct effect (CDE), natural indirect effect (NIE) and the total effect (TE; total effect = CDE + NIE) was considered. In detail, the CDE is the effect of education on sTNF-R1 when the considered mediators (BMI, WHR, WHeR) were conditioned on a fixed level (direct path: dashed arrow in Fig. 1) (30). In contrast, the NIE describes the change of sTNF-R1 blood levels if the educational level was fixed at a certain value, and the mediator would take the value caused by a change to the next higher educational level. This represents the indirect path (via mediator) displayed by undashed arrows in Fig. 1. Effect estimates refer to effect sizes independently from possible confounders (Table 1). The TE refers to the sum of both effects and thus describes the simple total association of education with inflammation after covariate adjustment.

We performed a sensitivity analysis to estimate the possible bias due to unmeasured confounders as proposed by Valeri and Vanderweele (30,31). In order to avoid possible distractions due to workload, a further sensitivity analysis assessed a possible mediation effect after excluding not retired subjects (359 men; 296 women). Additionally, we performed sensitivity analyses excluding retired subjects and subjects born after 1945 (remaining subjects received their education in the former German Democratic Republic).

The limit of statistical significance was assumed at an α of 5%. All statistical analyses and data management were performed using SAS® version 9.3 (SAS Inc., Cary, NC, USA). For the mediation analysis, we used the SAS macro compiled by Valeri and Vanderweele (30).

Missing values

The most missing values occurred in the case of inflammation parameters ranging between 7% (sTNF-R1) and 5% (hsCRP). Educational level and anthropometric parameters were recorded in all subjects. The minimal proportion of observations that entered the respective models was 87.8% (analysis of sTNF-R1). Using Mann–Whitney *U*-test/Student's *t*-test/chi-square test, there were no significant differences in educational level or the parameters taken as covariates into account between subjects with missing and without missing values. Thus, we conducted a complete case analysis.

Results

Descriptive results (Table 2)

Blood levels of sTNF-R1 were higher in male (1180.29; 95% confidence interval [CI]: 1149.74, 1211.65) than in female subjects (1047.19; 95% CI: 1019.9, 1075.2). Women had a lower blood pressure, consumed less alcohol and were less likely to be smokers, but reported more frequently to suffer from rheumatoid arthritis. Further parameters used in our analyses appeared to be of similar magnitude in men and women, and thus indicated no relevant sex differences.

Association analyses

Educational level

Education → Inflammation parameters (Table 3). Considerable inverse associations (high education associated with low chronic inflammation) occurred in the case of sTNF-R1 and hsCRP in female subjects in the unadjusted and adjusted models. In men, the association of education with sTNF-R1 was only significant after covariate adjustment. Again in men we found lower levels of hsCRP and IL-6 in subjects with higher education; however, the IL-6 association was only significant when the linear trend was considered (–14.3%/kg m^{-2}; 95% CI: –22, –5.8).

Education → Anthropometric parameters (Table 4). Examining the association between education and BMI, we found considerably lower values in higher education compared with the lowest reference group in univariate and adjusted regression models. When gender effects are considered, the effect of education on BMI seemed to be more distinct in female subjects when possible confounders were taken into account. The same was true for the WHeR, while there were virtually no considerable differences in WHR between the considered educational levels in men and women.

Table 2 Baseline characteristics

	Men Mean (95% confidence limits)	Women
Inflammation parameters		
sTNF-R1 (pg mL^{-1})	1180.29 (1149.74, 1211.65)	1047.19 (1019.9, 1075.2)
IL-6 (pg mL^{-1})	1.57 (1.47, 1.67)	1.63 (1.52, 1.74)
hsCRP (mg L^{-1})	2.06 (1.91, 2.22)	1.79 (1.65, 1.95)
Anthropometric parameters		
BMI (kg m^{-2})	28.1 (27.8, 28.3)	28.3 (27.9, 28.7)
WHR	1 (1, 1.01)	0.88 (0.88, 0.89)
WHeR	59.6 (59.2, 60)	59.3 (58.7, 59.9)
Further parameters		
Age	64.9 (64.2, 65.6)	63.7 (63, 64.4)
Systolic RR (mmHg)	146 (144.7, 147.3)	141.7 (140.1, 143.3)
Diastolic RR (mmHg)	86.1 (85.4, 86.8)	83.4 (82.6, 84.1)
GFR (CKD-EPI) (mL min^{-1}/1.73 m^2)	85.4 (84.3, 86.5)	85.3 (84.2, 86.4)
TSH (mU L^{-1})	0.75 (0.71, 0.8)	0.75 (0.7, 0.8)
Alcohol (g day^{-1})	1.98 (1.52, 2.57)	0.06 (0.04, 0.08)
Food index (30 = best/0 = worst)	14.51 (14.31, 14.72)	16.39 (16.17, 16.62)
Minimum/median/maximum		
Size of household	1/2/9	1/2/13
Health conviction – physical inactivity*	1/5/6	1/5/6
Health conviction – obesity/smoking*	1/6/6	1/6/6
Frequencies (*n* [%])		
Education: low/medium low/medium high/high	32 (3.5%)/370 (40.4%)/320 (34.8)/194 (21.2%)	111 (14.6%)/368 (48.4%)/212 (27.9%)/69 (9.1%)
Smoker (current; former; never)	203; 476; 240	112; 132; 516
Diabetes	142 (15.5%)	105 (13.8%)
Cancer	28 (3.1%)	8 (1.1%)
Rheumatoid arthritis	112 (12.2%)	153 (20.1%)
Bronchitis	68 (7.4%)	54 (7.1%)

*1: improves health status; 6: deteriorate health status.
BMI, body mass index; CKI-EPI, Chronic Kidney Disease Epidemiology Collaboration formula; GFR, glomerular filtration rate; hsCRP, high-sensitive C-reactive protein; IL-6, interleukin 6; RR, blood pressure; sTNF-R1, soluble tumour necrosis factor type 1; TSH, thyroid-stimulating hormone; WHeR, waist-to-height ratio; WHR, waist-to-hip ratio.

Table 3 Association analysis between educational level (continuous and categorized) and inflammation parameters

Gender	Education	sTNF-R1 (adj.)	hsCRP (adj.)	IL-6 (adj.)
Men	High	–18.6 (–29, –6.6)	–30.9 (–51.9, –0.8)	–32 (–55.9, 4.7)
	Medium high	–16.6 (–27, –4.8)	–18.1 (–42.3, 16.3)	–27.9 (–52.6, 9.6)
	Medium low	–14.8 (–25.3, –2.9)	–4.9 (–32.7, 34.2)	–8.6 (–39.5, 38.1)
	Low	(Reference)	(Reference)	(Reference)
	Continuous	–3.4 (–6.3, –0.5)	–13.8 (–20.3, –6.7)	–14.3 (–22, –5.8)
Women	High	–21.8 (–29.6, –13)	–40.9 (–56.6, –19.7)	–11.6 (–41.6, 33.8)
	Medium high	–13.9 (–20.6, –6.6)	–21.4 (–38, –0.5)	5.6 (–22.7, 44.3)
	Medium low	–8.5 (–15, –1.5)	–10.8 (–28.1, 10.8)	8.9 (–17.5, 43.7)
	Low	(Reference)	(Reference)	(Reference)
	Continuous	–7.3 (–10.1, –4.5)	–14.8 (–22, –6.9)	–3.4 (–14.3, 8.9)

Effect in percentage with 95% confidence intervals. Effect estimates refer to relative difference from reference category (lowest education) in percentage (categorized scale) and percentage change per increase in educational level (continuous scale). Adjusted for age, gender, neighbourhood and household size.
hsCRP, high-sensitive C-reactive protein; IL-6, interleukin 6; sTNF-R1, soluble tumour necrosis factor type 1.

Table 4 Association analysis between educational level (continuous and categorized) and anthropometric parameters

Gender	Education	BMI* (adj.)	WHR (adj.)	WHeR (adj.)
Men	High	–0.9 (–2.4, 0.7)	–0.02 (–0.04, 0)	–2.7 (–5, –0.4)
	Medium high	–0.3 (–1.8, 1.2)	–0.01 (–0.03, 0.01)	–1.6 (–3.8, 0.6)
	Medium low	0 (–1.5, 1.4)	0 (–0.02, 0.02)	–0.6 (–2.8, 1.6)
	Low	(Reference)	(Reference)	(Reference)
	Continuous	–0.4 (–0.7, 0)	–0.01 (–0.01, 0)	–0.9 (–1.5, –0.4)
Women	High	–2.9 (–4.6, –1.3)	–0.02 (–0.04, 0)	–4.7 (–7.2, –2.1)
	Medium high	–1.5 (–2.7, –0.3)	–0.01 (–0.02, 0.01)	–2.1 (–4, –0.1)
	Medium low	–0.4 (–1.5, 0.8)	0 (–0.02, 0.01)	–0.6 (–2.4, 1.1)
	Low	(Reference)	(Reference)	(Reference)
	Continuous	–1 (–1.5, –0.5)	–0.01 (–0.01, 0)	–1.5 (–2.2, –0.7)

Effect estimates with 95% confidence intervals. Effect estimates refer to relative difference from reference category (lowest education) in percentage (categorized scale) and absolute change per increase in educational level (continuous scale). Adjusted for age, gender, neighbourhood and household size.
*in kg m^{-2}.
BMI, body mass index; WHeR, waist-to-height ratio; WHR, waist-to-hip ratio.

Table 5 Association analysis between anthropometric and inflammation parameters

Gender	Education	sTNF-R1 (adj.)	hsCRP (adj.)	IL-6 (adj.)
Men	BMI (kg m^{-2})	7.4 (4.4, 10.4)	33.9 (24.8, 43.8)	11.7 (2.2, 21.9)
	WHR	6.7 (2.9, 10.6)	41.8 (29, 55.8)	2.8 (–8.3, 15.3)
	WHeR	8 (5, 11.2)	39.3 (29.5, 49.9)	9.5 (0, 20)
Women	BMI	10.6 (8.5, 12.8)	41.9 (34.1, 50.1)	19.7 (11.1, 29)
	WHR	7.2 (3.9, 10.7)	36.1 (24.2, 49.2)	7.4 (–4.3, 20.4)
	WHeR	9.4 (7.2, 11.6)	43.5 (35.6, 51.9)	18 (9.5, 27.2)

Effect estimates with 95% confidence intervals. Effect estimates refer to percentage change in inflammation parameters per standard deviation increase in anthropometric parameters. Adjusted for age, gender, bronchitis, currently smoked cigarettes, cigars, pipes, sport index (26), daily alcohol intake, presence of rheumatoid arthritis or cancer, thyroid-stimulating hormone, food index (27) and health conviction in terms of obesity.
BMI, body mass index; hsCRP, high-sensitive C-reactive protein; IL-6, interleukin 6; sTNF-R1, soluble tumour necrosis factor type 1; WHeR, waist-to-height ratio; WHR, waist-to-hip ratio.

Anthropometric parameters → Inflammation parameters (Table 5). The next step of the causal indirect chain is the association between anthropometric and inflammation parameters (second undashed arrow in Fig. 1). Here we found strong effects in male and female subjects for all anthropometric parameters taken into account. Estimates had a similar magnitude, while we observed a low variance in the case of sTNF-R1 leading to narrow confidence limits. Apart from its association with hsCRP in men (41.8%; 95% CI: 29, 55.8) WHR correlated most weakly with anthropometric parameters.

Mediation analysis (Table 6)

In the mediation analysis, we found that the BMI was a strong mediator of educational differences in sTNF-R1 and hsCRP (Table 6). Comparing both inflammation parameters, the effect was most pronounced when hsCRP was considered; here men and women showed a similar mediated amount of approximately one-third of the TE (NIE in men: –3%; 95% CI: –5.2, –0.7/TE in men: –8.5; 95% CI: –15.6, –0.8/NIE in women: –7.5; 95% CI: –11, –3.8/TE in women: –14.4%; 95% CI: –22, –6.1). Because of a weak TE mainly in men (NIE and TE combined less than 7%), the absolute mediation effect of BMI was lower in the case of sTNF-R1. Anthropometric parameters explained only a small fraction of the total education effect on IL-6 plasma levels in men. The inconclusive TE of education on IL-6 in female subjects makes it difficult to assess the indirect effect by BMI properly. WHeR had a similar mediation effect as BMI, while WHR was the weakest mediator in all analyses; however, strongest in the case of hsCRP in men (NIE: –2.6; 95% CI: –4.6, –0.5/TE: –8.7; 95% CI: –15.8, –1). Differences in the magnitude of the TE in the mediation analysis and the effect size in the association of education and

Table 6 Results of the mediation analyses of anthropometric parameters (effect estimates in percentage with 95% confidence limits)

Gender	Mediator	Effect	sTNF-R1	hsCRP	IL-6
Men	BMI	CDE	–1.8 (–4.7, 1.3)	–5.7 (–12.7, 2)	–12.4 (–20.5, –3.4)
		NIE	–0.7 (–1.3, –0.1)	–3 (–5.2, –0.7)	–1 (–2.2, 0.1)
		TE	–2.5 (–5.4, 0.6)	–8.5 (–15.6, –0.8)	–13.3 (–21.3, –4.4)
	WHR	CDE	–2 (–5, 1)	–6.3 (–13.3, 1.4)	–13.3 (–21.3, –4.3)
		NIE	–0.5 (–0.9, 0)	–2.6 (–4.6, –0.5)	–0.1 (–0.9, 0.8)
		TE	–2.5 (–5.5, 0.5)	–8.7 (–15.8, –1)	–13.3 (–21.3, –4.4)
	WHeR	CDE	–1.5 (–4.5, 1.5)	–4.7 (–11.8, 2.9)	–12.5 (–20.7, –3.5)
		NIE	–1 (–1.6, –0.3)	–4.1 (–6.5, –1.7)	–0.9 (–2.2, 0.4)
		TE	–2.5 (–5.4, 0.6)	–8.7 (–15.7, –1)	–13.3 (–21.3, –4.4)
Women	BMI	CDE	–4.8 (–7.7, –1.9)	–7.5 (–15.2, 0.9)	1 (–10.1, 13.4)
		NIE	–2.2 (–3.3, –1)	–7.5 (–11, –3.8)	–3.8 (–6.3, –1.3)
		TE	–6.9 (–9.8, –3.9)	–14.4 (–22, –6.1)	–2.9 (–13.5, 9.1)
	WHR	CDE	–6.7 (–9.6, –3.7)	–12.9 (–20.5, –4.6)	–2.8 (–13.4, 9.2)
		NIE	–0.3 (–0.8, 0.2)	–1.5 (–3.6, 0.7)	–0.3 (–1, 0.5)
		TE	–7 (–9.9, –3.9)	–14.2 (–21.8, –5.8)	–3 (–13.6, 8.9)
	WHeR	CDE	–5.3 (–8.2, –2.3)	–8 (–15.5, 0.3)	0.2 (–10.8, 12.5)
		NIE	–1.7 (–2.6, –0.7)	–6.8 (–10.5, –3)	–3 (–5.1, –0.8)
		TE	–6.9 (–9.8, –3.9)	–14.2 (–21.9, –5.9)	–2.8 (–13.4, 9.2)

Adjusted for neighbourhood, household size, age, gender, bronchitis, currently smoked cigarettes, cigars, pipes, sport index (26), daily alcohol intake, presence of rheumatoid arthritis or cancer, thyroid-stimulating hormone, food index (27), health conviction in terms of obesity, neighbourhood and household size.
BMI, body mass index; CDE, controlled direct effect; hsCRP, high-sensitive C-reactive protein; IL-6, interleukin 6; NIE, natural indirect effect; sTNF-R1, soluble tumour necrosis factor type 1; TE, total effect; WHeR, waist-to-height ratio; WHR, waist-to-hip ratio.

inflammation as described earlier are attributable to covariates taken into account.

Sensitivity analysis

In accordance with Valeri and Vanderweele (30), we assume an unmeasured binary confounder (e.g. a certain genotype or non-response) with an effect on inflammation plasma levels comparable with the amount of gender on sTNF-R1 blood levels (16.4% increase). With such a scenario to negate the indirect effect of BMI on hsCRP levels in women, the probability of having this confounder present had to be 46% higher by every increasing education level in women and 18% in men. In the case of the causal chain education-BMI-sTNF-R1 (lowest effect estimates), the probability of showing this unmeasured confounder had to rise by 13% in women and 4% in men. However, an unmeasured parameter with such a strong effect on inflammation and high prevalence in increasing/decreasing educational levels, respectively, is very unlikely, especially when the strong assumed effect of this potential confounder on inflammation is considered. Yet, such a confounding effect is most likely in men and sTNF-R1; however, it is still implausible as this confounder would be limited to this subgroup.

After considering retired subjects only, effect estimates and general findings were not relevantly altered. However, the mediating effect of WHR decreased considerably almost to the null effect estimate (see Table S1), which was most relevant in men when hsCRP was considered.

When we restricted the analyses to subjects born after 1945, effect estimates decreased in men, while we observed an increase of NIE estimates in women, both absolutely and relatively (see Table S5). As the subgroup with the lowest educational level exhibited a considerably lower plasma level of sTNF-R1 than the three remaining ones (Table 3), we performed mediation analyses with a binary factor of lowest educational level vs. the remaining subgroups. Here, we found a stronger direct and an almost unchanged indirect effect (equivalent to a relative decrease of the NIE in relation to the TE across anthropometric parameters), while CIs of the NIE became wider (see Table S6). This indicates that only (small) differences between higher levels of education could be considerably explained by anthropometric parameters related to obesity.

Discussion

Summarizing our findings, we revealed a considerable association of education with plasma levels of inflammation parameters in both sexes (i). Associations were generally stronger in women, which was however not true for IL-6, for which the relation was more distinct in men. About one-third of the TE of education on hsCRP plasma levels was mediated by BMI in both sexes (ii); however, the mediation effect by BMI appeared to be weaker in the

analyses of sTNF-R1 and IL-6. In more detail, both abdominal and hip circumferences seem to have a similar effect on the considered inflammation parameters. Additionally, waist circumference and body weight, but not WHR, were lower in subjects with higher education, resulting in an equally strong mediation effect of the WHeR and BMI, but not of WHR. Thus, we cannot assign a particular inflammation inducing effect to abdominal fat, but to obesity (iii) in general accounting for a maximum of just fewer than 50% on the total educational differences in inflammation.

Previous research confirmed a higher awareness to health issues and wider acceptance of scientific findings related to disease prevention and lifestyle in people with higher education (32). Although our cohort consisted of older subjects, the educational level appears to be a stable factor influencing health conviction and health status over several decades. This is supported by the sensitivity analysis, which showed that current workload could not explain the mediation effect.

Sex differences in the relation of educational level and inflammation might be explained by a higher health consciousness in women with advanced education. This reasoning is underpinned by a stronger association of educational level with anthropometric parameters in women compared with men. In contrast, IL-6 might be affected by parameters other than body weight that are related to education and are mostly prominent in men (such as smoking and alcohol consumption), as BMI explained only a small proportion of educational differences.

Nevertheless, the mediation of about two-thirds of the TE could not be explained by body weight, implicating further factors to be present. Closely linked to inflammation, in adolescence BMI seemed to mediate more than half of the total socio-economic effect on inflammation (18). However, in our collective of an elderly population, further factors such as psychosocial stress (32) and adverse life events (33) depending themselves on education might have an additionally mediating effect.

The considerable contribution of obesity in general to chronic inflammation in our cohort is surprising and partly in contrast to previous studies describing abdominal/visceral fat as the primary contributor to chronic inflammation (15–17,34). However, the WHR correlation with visceral fat mass was considerably weaker than with the WHeR or the BMI in a collective of the general population, limiting the usefulness of WHR to assess visceral fat mass (35). In more general, further data gave evidence for a relation of general obesity with low-grade chronic inflammation in both sexes (36) and in elderly women only (37). Using MRI data, a more recent study found that the subcutaneous but not the visceral fat was related to several inflammation parameters (19), while the production of soluble TNF receptors and IL-6 by subcutaneous fat was shown by another study (38). Summarizing previous findings, the most plausible explanation for the observed findings is that chronic inflammation is equally induced by visceral and subcutaneous fat tissue; however, the former is difficult to measure with the anthropometric parameters used in our study.

The weak association of education and IL-6 is in line with previous data from population-based samples (39), but in contrast to a further study (14). There is evidence that IL-6 activity might be linked to specific short-term stress reactions that cannot be captured by a constant parameter such as education (40). These specific pathways might be highly depended on characteristics of the examined collective such as age, workload and general life circumstances, which would explain the inconsistent results.

In summary, our findings show that education might be an important cause for inflammation-induced cardiovascular risk implicating the need and potential for risk reduction by education. An informed awareness of healthcare providers about medical disadvantages in lower social classes should also result in primary prevention geared to the needs of the respective high-risk groups.

Limitations

In the present study, we used a cross-sectional approach; however, prospective studies are needed to draw causal conclusions (32). Nevertheless, the long time period between the end of education and the onset of cardiovascular events makes it difficult to use a prospective approach when studying an elderly population. Other influencing factors becoming apparent during lifetime (such as a different workload and deviating adverse effects related to employment) might be in action, which makes a causal interpretation difficult. Nevertheless, a causal relation is more likely for the link between obesity-related parameters and inflammation as compared with the association of education with, for example, BMI, while a causal effect of education on obesity still seems more likely than the reverse. This leaves a certain amount of uncertainty in the actual causation of considered relations underlining the need for prospective studies. Here, our study can provide important information on effect sizes in the socio-economic assessment of key components of chronic inflammation.

Additionally, we could not quantify the amount of fat tissue in more detail as, for example, by imaging methods. Nevertheless, we would expect even stronger relations with a more accurate measurement method. Using plasma levels, we can only reveal relations regarding systemic inflammation; however, locally circumscribed inflammation might be different. Our results apply to the general population with the restriction of a collective above an age of 45 years. To generalize our findings on a wider scale, further studies are required which estimate effects in different collectives.

In conclusion, we found lower plasma levels of inflammation parameters related to subclinical chronic inflammation in (mainly female) subjects with higher education. This relation was mediated by anthropometric parameters of obesity.

Conflict of Interest Statement

All authors declare that there is no conflict of interest.

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Author contribution

DM wrote the paper/conducted the data analysis/corresponding author. HL revised the paper/analysed blood parameters/gave approval. AK revised the paper/organized the study/gave approval. DT revised the paper/organized the study/gave approval. SN conducted investigation/revised the paper/organized the study/gave approval. KHG revised the paper/organized the study/gave approval. JT revised the paper/organized the study/gave approval. KW revised the paper/organized the study/gave approval. JH revised the paper/organized the study/gave approval. BH revised the paper/organized the study/participated in the data analysis/gave approval.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Table S1. Results of the mediation analyses of anthropometric parameters after exclusion of non-retired subjects (effect estimates in percentage with 95% confidence limits).

Table S2. Association analysis between educational level (continuous and categorized) and inflammation parameters. Effect in percentage with 95% confidence intervals. Unadjusted and adjusted effect estimates.

Table S3. Association analysis between educational level (continuous and categorized) and anthropometric parameters. Effect estimates with 95% confidence intervals. Unadjusted and adjusted effect estimates.

Table S4. Association analysis between anthropometric and inflammation parameters. Effect estimates with 95% confidence intervals. Unadjusted and adjusted effect estimates.

Table S5. Results of the mediation analyses of anthropometric parameters after excluding subjects born before 1946 (effect estimates in percentage with 95% confidence limits).

Table S6. Results of the mediation analyses of anthropometric parameters after modelling the effect of the subgroup with the lowest education vs. the remaining subgroups (effect estimates in percentage with 95% confidence limits).

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Educational status and differences in left ventricular mass and ejection fraction – The role of BMI and parameters related to the metabolic syndrome: A longitudinal analysis from the population-based CARLA cohort

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Mediation analysis

Abstract *Background and aims:* Higher ventricular mass has been reported in non-white US-Americans with low educational status and in socially isolated people. To assess the impact of education on cardiac mass and function in the general population and to identify mediators.
Methods and results: Data from a German population-based sample were used (CARLA cohort, n = 1779 at baseline, n = 1436 at the four-year follow-up). Ventricular mass indexed on height (LVMI) and ejection fraction, using Teichholz's formula (EFTZ), were measured. Education was assessed using the ISCED classification. Mediator analyses were performed using the R-macro 'mediation' to compute the average direct effect and the average causal mediated effect after confounder adjustment. Sensitivity analyses for unobserved confounders were performed. Considered mediators were BMI, waist-to-hip ratio, HbA1c, and systolic and diastolic blood pressures.

We found differences in LVMI and EFTZ, both at baseline and follow-up, between educational levels in women (lowest vs highest educational level: 15.6 g, 95% CI: −25.7, −5.6), but not in men. Similarly, women (lowest vs highest educational level at baseline: 3.3%, 95% CI: 0.8–5.7), but not men, of higher educational levels had a higher EFTZ of comparable magnitude at baseline and follow-up. Of the considered mediators, BMI explained 55.9% at baseline and 54.1% at follow-up of the educational effect, while other potential mediators had no significant effect. Relations remained constant between baseline and follow-up.
Conclusions: Women with low educational levels tend to have a higher ventricular mass and lower EF, which can be explained by a higher BMI in this group.

Introduction

In the early 1990s, data from the Framingham Heart Study provided overwhelmingly strong evidence for the value of echocardiographically measured ventricular mass in the prediction of cardiovascular events in addition to established risk factors [1,2]. Focussing on social aspects, our group reported previously that lower social classes accumulate cardiovascular risk factors [3], which are mainly due to an adverse lifestyle (smoking, obesity and low physical activity) [4]. However, the explanatory effect

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of these factors on differences in cardiac mass or function between groups with different educational status remains largely unknown.

Data from the United States indicate a higher left ventricular mass in socially isolated subjects [5] due to different habits in smoking and physical activity. In an earlier US-American study, left ventricular mass was higher among non-white subjects with a low socioeconomic status, while similar inverse relations between SES and left ventricular mass could not be shown for whites [6]. The factors (mediators, as they are called in epidemiological studies) that contribute to these social differences remain unknown. Parameters related to the metabolic syndromes are most likely to be the key mediators, and most prominent among them is obesity. In several studies, low socioeconomic status is related to higher weight and an increased prevalence of obesity [7]. Surprisingly, according to population-based data, this relation is more pronounced in women than in men [8,9].

Thus, the current study examines a sample of the general population with the aim of detecting the influence of educational levels on ventricular mass and systolic function. As a second step, the present study also aims to assess the mediating effect of metabolic factors on the potential differences. To our knowledge no previous study has assessed differences in cardiac mass and function, respectively, in a cross-sectional and longitudinal design or has estimated the mediating effect of cardiovascular risk factors on such disparities using refined methods of causal inferences.

Methods

Study cohort

We used data from the Cardiovascular Disease, Living and Ageing in Halle (CARLA) study, which is a prospective population-based cohort study of the elderly general population of the city of Halle in eastern Germany [10]. The CARLA cohort comprises 1779 participants (baseline response 64.1%), aged 45–83 years at baseline (967 men, 812 women). The baseline examination took place between December 2002 and January 2006. A multi-step recruitment strategy aimed to achieve a high response rate. The final response rate after subtracting exclusions (individuals who were deceased prior to the invitation, had moved away, or were unable to participate due to illness) was 64%. From March 2007 until March 2010 (mean follow-up time: 4.01 years), the first four-year follow-up examination was performed. The net sample (after exclusion of deceased or non-responding people) then comprised 1436 subjects (86% response rate), consisting of 790 men and 646 women aged between 50 and 87 years, which were taken into account for analyses. The study participants underwent a detailed medical examination and a standardized, computer-assisted interview, which collected information on socio-demographic and education-related variables, behavioural, biomedical and psychosocial factors, medical history, and the use of medication within the preceding seven days. Additionally, an analysis of non-respondents was performed in order to assess non-response bias. A more comprehensive description of the CARLA study can be found in Greiser et al. [10,11]. The study was approved by the Ethics Committee of the Medical Faculty of the Martin Luther University Halle-Wittenberg and by the State Data Privacy Commissioner of Saxony-Anhalt, and conforms to the principles outlined in the Declaration of Helsinki [12]. All participants gave written informed consent.

Echocardiographic assessment

At baseline, Doppler echocardiographic examinations were conducted and evaluated by a specially trained and certified physician. At follow-up, echocardiography was performed by a trained and certified study nurse, and the stored echocardiographic recordings were subsequently evaluated by a trained physician. All echocardiographers underwent the same dedicated study certification procedures. Assessing intra-observer variability for the M-mode examinations, the mean observer bias varied between 0.3% and 3.8% (2*SD between 15.3% and 27.7%), while the inter-observer variability ranged between 0.1% and 2.7% (2*SD between 12.7% and 20.8%). All echocardiographic examinations at baseline and follow-up were performed using the GE Vivid ultrasound system (GE Vivid 4 and 5 at baseline, GE vivid 5 at follow-up). We took only dimensional parameters, rather than volume parameters, into account. To calculate the left ventricular mass index (LVMI), we used the ASE-cube formula divided by body height, which is in accordance with current recommendations [13]. The cardiac output as a functional parameter was measured by the ejection fraction according to Teichholz (EFTZ) [14].

Educational level

Educational level was defined in accordance with the International Standard Classification of Education (ISCED), version 1997 [15], assessing years of education in accordance with a standardized algorithm including school, job and academic degree. Categories were formed as follows, with the indication of the years of education in brackets: low (9–10 years), medium low (11–13 years), medium high (14–17), and high (18–20 years). Education refers to status during the baseline examination.

Further parameters

Information about smoking habits, medication, presence or history of diseases and daily alcohol intake was investigated using a computer-based interview and subsequently reviewed by a physician. Blood pressure is the mean of three consecutive measures after a resting time of at least 5 min.

Statistical methods

Mixed models were used to examine the association of education (categorized and linear trend) with LVMI and EFTZ, adjusting for age and neighbourhood (considered as a random factor). Variable selection and modelling was based on directed acyclic graphs [16]. Investigating the baseline to follow-up change in LVMI or EFTZ, we considered the individual annual change in these parameters as the outcome variable. When we adjusted our models for the probability (predictors were age, sex, comorbidity, BMI) of missingness at baseline and follow-up, estimates remained virtually unaltered; thus, we conducted a complete case analysis (missing EFTZ: 5.8%, missing LVMI: 4.8% at baseline).

Mediation analysis

For the mediation analysis, we used the R-package 'mediation' [17]. As effect measures, the average direct effect (ADE), the average causal mediation effect (ACME) and the total effect (TE, total effect = ADE + ACME) were considered.

In short, the ADE is the effect of education on ventricular mass or ejection fraction when the considered mediators were conditioned on a fixed level (direct path: dashed arrow in Fig. 1) [18]. In contrast, the ACME describes the change of ventricular mass or ejection fraction if the educational level was fixed at a certain value, and the mediator would take the value caused by a change to the next higher educational level. This represents the indirect path (via mediator) displayed by a drawn-through arrow in Fig. 1. A more comprehensive description and derivation of causal effects implemented in the used R package can be found in Imai et al. or Valeri/Vanderweele [18,19].

We adjusted for age and neighbourhood as potential confounders of the association of education and parameters of metabolic syndrome (pretreatment covariates, confounders 1 in Fig. 1). Confounders of the association of the latter and ventricular mass or ejection fraction are likely to be affected by educational level and thus have the additional character of a mediator. This leads to the problem of multiple mediators that are causally dependent. The 'multimed' function in R allows for the adjustment of these multiple mediators during the modelling process and to estimate the mediating effect of one main confounder (confounders 2 in Fig. 1) independently from other mediators. Potential confounders, which need to affect metabolic parameters and cardiac structure or function independently from BMI in order to be considered as confounders of the mediation analysis, were sport index [20], daily smoked cigarettes, thyroid medication, hyperthyreosis, and BMI (only when BMI was not the main mediator).

In the case of multiple causally related mediators, the interpretation of the ACME extends slightly, as the effect of these alternative mediators on the main mediators needs to be taken into account. In this case, the ACME is the outcome change when the educational level remains fixed but the main mediator and the alternative mediator, which is causally related to the main mediator, would take the value caused by an increase of the educational variable by one unit.

As potential mediators, we took BMI, waist-to-hip ratio (WHR), HbA1c, and systolic and diastolic blood pressures into account, which are related to the metabolic syndrome. Diabetes mellitus was only assessed as reported by the subject, rather than by more objective methods such as the oral glucose tolerance test. This is an important limitation when mediation analyses are performed; thus, we refrain from using diabetes mellitus as a possible mediator and only report the results considering diabetes occasionally in the main text, if applicable. For the mediation analyses, we considered all educational levels, and in another analysis, we considered combined levels with a similar difference in respect to the reference level to take the factorial character of education into account (see grouping in the Results section).

In cases where we found significant differences between educational classes and cardiac mass, mediation analyses will be performed in men and women and for the baseline examination, which will be contrasted to the follow-up.

Sensitivity analyses

We performed sensitivity analyses to estimate the impact of a violation of the homogeneous interaction (constant interaction of the main mediator and educational level across individual levels) assumption by

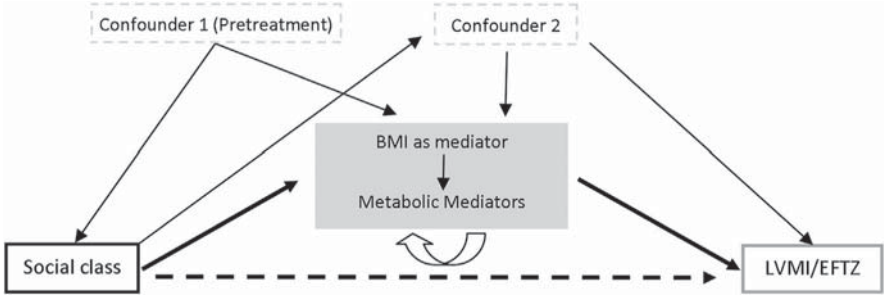

Figure 1 Directed acyclic graphs of causal relations assumed in the mediation analysis. The wide unfilled arrow illustrates the interdependency of considered metabolic mediators. Grey shaded box: mediators.

estimating the amount of variance of the outcome variable that would need to be explained if this assumption was violated [21].

However, in the case of a statistically significant mediator, we assessed the robustness of the effect towards unobserved confounders, as proposed by Imai et al. [17,18], with the other multiple mediators treated as confounders (rather than mediators) and, thus, constraining the earlier model when they were permitted to be influenced by education.

To assess the impact of a potentially confounding effect of height, sensitivity analysis was performed where we adjusted for height as a pre-treatment variable. Additionally, we performed a further sensitivity analysis where we used the biplane Simpson's method to assess ejection fraction, which was, however, only available at follow-up. Here effect estimates of the mediation analysis using the Teichholz formula are compared to those found when Simpson's approach was used.

The limit of statistical significance was assumed at a α of 5%. All statistical analyses and data management were performed using SAS®, Version 9.3 (SAS Inc., Cary, NC, USA) and R 3.1.2 [22].

Results

Basic characteristics of the CARLA cohort are given in Table 1, illustrating sex difference and alteration between baseline and follow-up. Blood pressure was common in the cohort with a proportion of circa three quarter which was slightly higher in men than in women.

Association of education with cardiac mass at baseline and follow-up (Table 2)

In men, mean LVMI differed only slightly between educational levels at baseline, while differences appeared more pronounced at follow-up. Analyzing average EFTZ, there were virtually no relevant differences between educational levels, both at the baseline investigation and at follow-up in men.

However, in women we found considerable educational differences in LVMI and EFTZ, both at baseline and at follow-up. The two groups with the highest level of education had an EFTZ that was 3.4% (95% CI: 1.6−5.3) and 3.3% (95% CI: 0.8−5.7), respectively, higher when compared with subjects with low education levels. These differences in EFTZ were more pronounced at the follow-up investigation.

Similarly, both groups with the most advanced education level had an LVMI that was 14.3 g/m (95% CI: −22.0, −6.6) and 15.6 g/m (95% CI: −25.7, −5.6), respectively, lower than in the reference group, while the remaining group ranged in between. At follow-up, the difference in LVMI between the lowest and highest educational levels increased to 22.1 g/m (95% CI: −35.4, −8.8).

Mediator analysis (Table 3a)

Focussing on the educational differences in LVMI in women, BMI explained more than 50% of the social differences observed. In detail, the LVMI was estimated to decrease by 6.1 g/m (95% CI: 3.3, 9.7) per educational level, and of this, BMI explained −3.4 g/m (95% CI: −5.0, −1.8) or 55.9%. At follow-up, the proportion explained by BMI was virtually unaltered with an estimated value of 54.1% (Table 4a), indicating the temporal durability of the BMI-mediated effect. Other metabolic indices could not explain a similar extent of educational differences in LVMI.

Regarding EFTZ, no metabolic index was able to explain a relevant and statistically significant amount of the SES when BMI was incorporated as a causal alternative mediator in the model. This was especially true for self-reported diabetes mellitus, which explained less than 10% of the total effect and did not reach statistical significance (results not shown).

Table 1 Basic characteristics.

	Baseline		Follow-up	
	Men	Women	Men	Women
LVMI (g/m)	123.96 [121.84, 126.09]	108.66 [106.66, 110.66]	118.51 [115.91, 121.11]	100.54 [98.04, 103.04]
EFTZ (%)	0.62 [0.61, 0.62]	0.65 [0.65, 0.66]	0.65 [0.65, 0.66]	0.68 [0.67, 0.69]
BMI (g/m^2)	28.15 [27.89, 28.41]	28.54 [28.17, 28.91]	28.2 [27.92, 28.49]	28.54 [28.12, 28.96]
WHR	1.00 [1.00, 1.01]	0.88 [0.88, 0.89]	1.01 [1, 1.01]	0.87 [0.86, 0.87]
HbA1c (%)[b]	5.71 [5.66, 5.76]	5.69 [5.63, 5.74]	5.83 [5.78, 5.88]	5.82 [5.77, 5.87]
Systolic RR (mmHg)	145.86 [144.62, 147.11]	141.71 [140.15, 143.28]	138.75 [137.41, 140.08]	135.17 [133.62, 136.72]
Diastolic RR (mmHg)	85.91 [85.21, 86.62]	83.22 [82.47, 83.96]	80.59 [79.85, 81.33]	79.05 [78.3, 79.79]
Education (minimum/ median/maximum)	1/3/4	1/2/4	1/2/4	1/2/4
Hypertension[a]: hypertensive subjects (total sample)/proportion with 95% confidence intervals	795 (967)/82.2% [79.7, 84.6]	611 (812)/75.2% [72.1, 78.2]	626 (786)/79.6% [76.7, 82.4]	493 (640)/77.0% [73.6, 80.2]

Mean with 95% confidence intervals for continuous variables.
Abbreviations: LVMI: left ventricular mass index (indexed on height), EFTZ: ejection fraction (Teichholz), BMI: body mass index, WHR: waist-to-hip ratio, RR: blood pressure.
[a] Hypertension: Blood pressure > 140/90 mmHg or antihypertensive drugs, exact confidence limits.
[b] Geometric mean.

Table 2 Mean differences in left ventricular mass (indexed on height) and ejection fraction (Teichholz) in relation to educational level.

Educational status	Sex	Left ventricular mass/height		Ejection fraction (Teichholz)	
		Crude	Adjusted	Crude	Adjusted
Baseline					
Low	Men				
Medium low	Men	3.2 [−11.0, 17.3]	2.6 [−11.2, 16.3]	−0.6 [−3.8, 2.6]	−0.6 [−3.7, 2.6]
Medium high	Men	−0.8 [−15.0, 13.4]	−4.1 [−18.0, 9.8]	−0.6 [−3.8, 2.6]	−0.2 [−3.4, 3.0]
High	Men	−0.3 [−15.0, 14.3]	−2.6 [−16.8, 11.7]	1.4 [−1.9, 4.7]	1.7 [−1.6, 4.9]
Low	Women				
Medium low	Women	−10.0 [−17.2, −2.9]	−4.8 [−11.9, 2.3]	2.2 [0.5, 3.9]	2.1 [0.4, 3.9]
Medium high	Women	−21.1 [−28.8, −13.4]	−14.3 [−22.0, −6.6]	3.5 [1.7, 5.3]	3.4 [1.6, 5.3]
High	Women	−24.7 [−34.7, −14.6]	−15.6 [−25.7, −5.6]	3.4 [1.0, 5.8]	3.3 [0.8, 5.7]
Follow-up					
Low	Men				
Medium low	Men	−8.0 [−26.8, 10.9]	−9.8 [−28.5, 9.0]	−0.6 [−5.5, 4.3]	−0.4 [−5.3, 4.5]
Medium high	Men	−9.1 [−28.0, 9.8]	−12.5 [−31.3, 6.4]	−0.2 [−5.1, 4.7]	0.1 [−4.8, 5.0]
High	Men	−6.0 [−25.3, 13.2]	−8.8 [−27.9, 10.3]	1.1 [−3.9, 6.0]	1.3 [−3.7, 6.3]
Low	Women				
Medium low	Women	−12.1 [−22.3, −2.0]	−9.1 [−19.3, 1.2]	3.2 [0.1, 6.3]	3.6 [0.4, 6.7]
Medium high	Women	−18.3 [−28.8, −7.7]	−14.7 [−25.4, −4.0]	3.7 [0.6, 6.9]	4.1 [0.9, 7.4]
High	Women	−26.7 [−39.8, −13.6]	−22.1 [−35.4, −8.8]	4.3 [0.3, 8.2]	4.8 [0.7, 8.8]
Baseline to follow-up change					
Low	Men				
Medium low	Men	−3.7 [−8.4, 1.0]	−3.6 [−8.3, 1.1]	−0.6 [−6.1, 4.9]	−0.6 [−6.1, 4.9]
Medium high	Men	−4.2 [−8.9, 0.6]	−3.9 [−8.7, 0.8]	0.8 [−4.7, 6.3]	0.8 [−4.7, 6.3]
High	Men	−4.2 [−9.0, 0.6]	−4.0 [−8.8, 0.8]	0.7 [−4.8, 6.3]	0.7 [−4.9, 6.3]
Low	Women				
Medium low	Women	−0.6 [−3.0, 1.9]	−0.9 [−3.4, 1.6]	0.2 [−3.0, 3.5]	0.4 [−2.9, 3.7]
Medium high	Women	−0.1 [−2.6, 2.5]	−0.5 [−3.1, 2.1]	−0.1 [−3.5, 3.3]	0.1 [−3.4, 3.5]
High	Women	−0.6 [−3.8, 2.5]	−1.2 [−4.4, 2.0]	0.5 [−3.7, 4.7]	0.7 [−3.6, 5.0]

Mean differences with 95% confidence intervals in relation to the group with the lowest educational level.

Mediator analysis – grouping (Table 3b)

Educational differences in LVMI were similar between the two highest classes. When they were combined into one, differences between the levels became slightly more pronounced, while the proportion explained by BMI remained virtually constant. At follow-up, educational differences increased homogeneously between the four levels of education, making no further merging necessary (Table 4b).

Regarding EFTZ, the two highest groups of education level had similar differences when compared with the group of lowest education level. Again, when they were summarized, values were more pronounced while BMI again explained more than 50% of the total effect. Interestingly, when we merged both groups with the highest educational levels at baseline, we found an intermediate mediation effect by BMI on educational differences in EFTZ. Between the lowest, the second lowest and the two remaining groups, the difference in EFTZ was estimated to be 1.5% (95% CI: 0.8–2.4), and of this, BMI mediated 0.4 (95% CI: 0.1–0.8), which is equivalent to 29.0%.

Sensitivity analysis

LVMI

Considering the sensitivity analysis of the homogeneous interaction assumption, we found that relaxing this assumption had to explain 14% of the total LVMI variance to eliminate the BMI-mediated effect. Using simple linear

Table 3a Mediation effect of parameters related to the metabolic syndrome in women at baseline.

Parameters	BMI	WHR	HbA1c	Systolic RR	Diastolic RR
LVMI					
ACME	−3.4 [−5.0, −1.8]	−0.4 [−1.1, 0.3]	0.1 [−0.5, 0.7]	−0.5 [−1.3, 0.3]	0.2 [−0.4, 0.7]
ADE	−2.8 [−5.8, 0.3]	−5.7 [−9.4, −2.0]	−6.4 [−9.6, −3.3]	−5.6 [−8.6, −2.7]	−6.3 [−9.7, −2.8]
Total effect	−6.1 [−9.7, −3.3]	−6.1 [−9.7, −3.0]	−6.2 [−10.1, −4.1]	−6.1 [−9.3, −2.9]	−6.1 [−9.2, −2.6]
EFTZ					
ACME	0.2 [−0.0, 0.4]	0.1 [−0.1, 0.2]	0.1 [−0.1, 0.3]	0.0 [−0.2, 0.2]	0.1 [−0.1, 0.2]
ADE	1.0 [0.2, 1.8]	1.1 [0.4, 1.8]	1.1 [0.3, 1.8]	1.1 [0.4, 1.9]	1.1 [0.4, 1.8]
Total effect	1.1 [0.4, 1.8]	1.1 [0.5, 1.9]	1.2 [0.5, 1.9]	1.1 [0.5, 1.9]	1.1 [0.6, 1.9]

The ADE is the part of the association of education with LVMI and EFTZ, respectively that cannot be explained by the considered mediator, while ACME is the amount of this association that is mediated (95% confidence intervals in brackets).
Abbreviations: ADE: average direct effect, ACME: average causal mediated effect, LVMI: left ventricular mass index (indexed on height), EFTZ: ejection fraction (Teichholz), BMI: body mass index, WHR: waist-to-hip ratio, RR: blood pressure.

Table 3b Mediation effect of parameters related to the metabolic syndrome after merging groups with similar mean values in women at baseline.

Parameters	BMI	WHR	HbA1c	Systolic RR	Diastolic RR
LVMI					
ACME	−3.9 [−6.4, −1.4]	−0.5 [−1.9, 0.9]	−0.5 [−1.9, 0.9]	−1.0 [−2.4, 0.4]	−0.2 [−1.6, 1.2]
ADE	−3.7 [−7.5, 0.1]	−7.0 [−11.1, −2.9]	−7.3 [−11.2, −3.4]	−6.6 [−10.5, −2.8]	−7.4 [−11.8, −3.0]
Total effect	−7.6 [−11.0, −3.3]	−7.6 [−11.7, −4.3]	−7.7 [−11.1, −4.1]	−7.6 [−11.1, −4.3]	−7.6 [−11.4, −3.5]
EFTZ					
ACME	0.4 [0.1, 0.8]	0.3 [−0.1, 0.7]	0.3 [−0.1, 0.7]	0.2 [−0.3, 0.6]	0.3 [−0.1, 0.7]
ADE	1.1 [0.3, 1.9]	1.2 [0.2, 2.2]	1.2 [0.3, 2.1]	1.3 [0.4, 2.2]	1.2 [0.4, 2.0]
Total effect	1.5 [0.8, 2.4]	1.5 [0.7, 2.3]	1.5 [0.6, 2.5]	1.5 [0.7, 2.5]	1.5 [0.8, 2.3]

For LVMI and EFTZ the educational levels 'medium high' and 'high' (linear comparison: 1–2–3 and 4) were merged because of similar means in relation to the reference (see Table 2).
The ADE is the part of the association of education with LVMI and EFTZ, respectively that cannot be explained by the considered mediator, while ACME is the amount of this association that is mediated (95% confidence intervals in brackets).
Abbreviations: ADE: average direct effect, ACME: average causal mediated effect, LVMI: left ventricular mass index (indexed on height), EFTZ: ejection fraction (Teichholz), BMI: body mass index, WHR: waist-to-hip ratio, RR: blood pressure.

Table 4a Mediation effect of parameters related to the metabolic syndrome in women at follow-up.

Parameters	BMI	WHR	HbA1c	Systolic RR	Diastolic RR
LVMI					
ACME	−2.6 [−4.2, −0.9]	−1.1 [−2.2, −0.1]	−0.3 [−1.2, 0.7]	−0.7 [−1.9, 0.5]	−0.3 [−1.2, 0.5]
ADE	−2.2 [−5.9, 1.5]	−3.6 [−7.5, 0.4]	−4.5 [−8.5, −0.5]	−4.0 [−7.7, −0.3]	−4.4 [−8.1, −0.7]
Total effect	−4.7 [−8.6, −1.4]	−4.7 [−8.2, −1.1]	−4.7 [−8.8, −1.7]	−4.7 [−8.5, −1.6]	−4.7 [−8.4, −1.5]
EFTZ					
ACME	0.0 [−0.4, 0.4]	−0.1 [−0.4, 0.3]	−0.1 [−0.5, 0.4]	−0.1 [−0.5, 0.3]	0.0 [−0.3, 0.3]
ADE	1.0 [−0.3, 2.2]	1.1 [−0.1, 2.3]	1.1 [−0.3, 2.4]	1.1 [0.0, 2.2]	1.0 [−0.1, 2.2]
Total effect	1.0 [0.1, 2.4]	1.0 [0.0, 2.2]	1.0 [−0.5, 2.0]	1.0 [0.0, 2.1]	1.0 [−0.2, 2.0]

The ADE is the part of the association of education with LVMI and EFTZ, respectively that cannot be explained by the considered mediator, while ACME is the amount of this association that is mediated (95% confidence intervals in brackets).
Abbreviations: ADE: average direct effect, ACME: average causal mediated effect, LVMI: left ventricular mass index (indexed on height), EFTZ: ejection fraction (Teichholz), BMI: body mass index, WHR: waist-to-hip ratio, RR: blood pressure.

regression analyses, we found that the interaction term explained less than 1% of the total variance of LVMI, indicating a strong robustness of our results against a possible violation of the assumption of interaction homogeneity.

Checking for unobserved confounders, we found that: (1) the potential confounder needs to affect ventricular mass and BMI in opposite directions (increasing one and decreasing the other), and (2) the product of the amount of variances (ventricular mass and BMI) explained by this confounder needs to be greater than 0.168 to eliminate the mediation effect by BMI (e.g. the confounder would need to contrarily explain 50% of the variance of LVMI and, simultaneously, 34% of the variance of BMI, or vice versa). Considering the fact that age explained only 6% of the total LVMI variance, it is very unlikely that an unobserved

Table 4b Mediation effect of parameters related to the metabolic syndrome after merging groups with similar mean values in women at follow-up.

Parameters	BMI	WHR	HbA1c	Systolic RR	Diastolic RR
LVMI					
ACME	–	–	–	–	–
ADE	–	–	–	–	–
Total effect	–	–	–	–	–
EFTZ					
ACME	0.0 [−0.2, 0.2]	0.0 [−0.2, 0.2]	0.0 [−0.3, 0.3]	0.1 [−0.2, 0.3]	0.1 [−0.2, 0.3]
ADE	0.3 [−2.2, 1.5]	−0.3 [−2.1, 1.5]	−0.4 [−2.4, 1.7]	−0.4 [−2.5, 1.7]	−0.4 [−2.3, 1.5]
Total effect	−0.3 [−2.1, 1.7]	−0.3 [−2.5, 0.8]	−0.3 [−2.3, 1.4]	−0.3 [−2.3, 1.4]	−0.3 [−1.9, 1.4]

For LVMI and EFTZ the educational levels 'medium high' and 'high' (linear comparison: 1, 2, 3–4) were merged because of similar means in relation to the reference (see Table 2). For LVMI, the model which took educational status as a linear predictor into count was found to be a good fit to the mean values.
The ADE is the part of the association of education with LVMI and EFTZ, respectively that cannot be explained by the considered mediator, while ACME is the amount of this association that is mediated (95% confidence intervals in brackets).
Abbreviations: ADE: average direct effect, ACME: average causal mediated effect, LVMI: left ventricular mass index (indexed on height), EFTZ: ejection fraction (Teichholz), BMI: body mass index, WHR: waist-to-hip ratio, RR: blood pressure.

confounder with such strong relations to LVMI and BMI was missed in our sample.

EFTZ

The BMI-mediated effect on EFTZ is again unlikely to be altered by an unobserved confounder, as the product of the proportion of BMI-explained EFTZ variance and the proportion of explained variance due to this confounder had to exceed 0.36 when compared with the previous sensitivity analysis when LVMI was the outcome.

Adjusting for height as a pre-treatment confounder led to virtually unaltered effect estimates, underlining the robustness of our findings against the impact of height (results not shown). Similarly, when we used the biplane Simpson's method (measured at follow-up) to assess ejection fraction effect estimates were almost unchanged when compared with the analyses using the Teichholz formula (Table 5).

Discussion

In summary, we found that educational differences influence ventricular mass and function in women of a representative sample of the general population. These differences were consistent during the observational time of four years between the baseline and follow-up investigation. In men, we could not determine similar differences in cardiac mass and function depending on education; although education-dependent differences became stronger at follow-up, wide confidence intervals do not allow us to determine a clear baseline-to-follow-up change.

Trying to explain educational differences in ventricular mass by mediators related to the metabolic syndrome, BMI is the outstanding parameter, mediating more than 50% of this educational heterogeneity. Waist circumference, apart from BMI, showed no evidence of a mediating role concerning social differences in ventricular mass. It might be concluded that abdominal fat, with its strong inflammatory effect, might play a lesser role than general obesity. However, it remains doubtful whether the mere waist circumference is an appropriate way to measure abdominal fat. Here, the accumulation of error terms (abdominal fat represented by waist circumference, measurement of waist circumference) might hamper the revealing of such subtle relations. However, it can be concluded from our data that waist circumference is far inferior to BMI when one tries to explain educational differences in cardiac mass. As the sensitivity analyses revealed, this effect is unlikely to be confounded by unmeasured parameters. BMI explained part of the lower EFTZ observed in female subjects with low education levels, although the mediation was weaker than in the analyses when LVMI was the outcome. Remarkably, this effect was very robust against a possible mitigation due to unobserved confounders. The possibility that the mentioned relations are confined to women needs to be considered.

Referring to the findings from the Framingham Heart Study, women, compared to men, have a higher cardiovascular risk due to an increased ventricular mass. Applying their findings to our collective, women in the group with the lowest educational level have a 26.4% (95% CI: 8.0–47.7%) higher risk for cardiovascular death due to a higher ventricular mass than their better-educated counterparts (highest level). However, the prognostic value of ejection fraction differs between studies [23–25]. Devereux et al. [23] reported a relative risk of 2.9 (95% CI: 1.6–5.4) for cardiovascular death when subjects had an ejection fraction below 55%. In our cohort, subjects of the lowest educational level were about twice as likely to have an EF below this threshold than subjects with the highest level.

During the four-year observational period, there was no evidence for an effect mitigation of this adverse relation; to the contrary, we observed a slight increase in effect differences between educational levels. The increase was stronger in men than in women. Eventually, we would expect even larger differences, as seriously affected subjects, who are more likely to descend from classes with low education levels, have a tendency for a higher drop-out rate. Indeed, we observed a higher proportion of subjects from lower educational classes who did not show up for the follow-up (results not shown).

Though our cohort consisted of older subjects, the educational level appears to be a stable factor influencing health consciousness and health status over several decades and even continues to augment as people grow older. Similarly, BMI seems to be a temporally constant factor given the fact that structural cardiac alterations need several years to develop. For primary prevention, this underlines the need to focus on obese elderly with limited education. When considering primary prevention, our results also show that sportive behaviour (the sport index proposed by Baecke et al. [20] was considered as a covariate in our mediator model) does not suffice to negate the BMI-induced cardiac alterations, but a further lifestyle adaption is likely to be necessary.

Table 5 Mediation effect of parameters related to the metabolic syndrome in women at follow-up taking the ejection fraction according to Simpson into account.

	BMI	WHR	HbA1c	Systolic RR	Diastolic RR
ACME	0.0 [−0.3, 0.4]	−0.1 [−0.4, 0.2]	0.0 [−0.2, 0.3]	0.0 [−0.3, 0.3]	0.0 [−0.2, 0.3]
ADE	1.0 [−0.3, 2.3]	−0.2 [−1.1, 0.6]	−0.3 [−1.2, 0.6]	−0.3 [−1.1, 0.5]	−0.3 [−1.2, 0.6]
Total effect	1.0 [−0.2, 2.3]	−0.3 [−1.3, 0.4]	−0.3 [−1.1, 0.8]	−0.3 [−1.3, 0.6]	−0.3 [−1.2, 0.5]

The ADE is the part of the association of education with LVMI and EFTZ, respectively that cannot be explained by the considered mediator, while ACME is the amount this association that is mediated (95% confidence intervals in brackets).
Abbreviations: ADE: average direct effect, ACME: average causal mediated effect, EFTZ: ejection fraction (Simpson), BMI: body mass index, WHR: waist-to-hip ratio, RR: blood pressure.

Further parameters had only weak explanatory power and, similarly, we could not disclose any statistical evidence for these parameters to be mediators of educational differences in LVMI or ejection fraction. It is remarkable that blood pressure was not a BMI-independent mediator of educational differences in cardiac mass and function, although increased blood pressure is a major trigger of (concentric) cardiac hypertrophy.

Comparing our findings to previous studies, they fit well into the framework of increased left ventricular mass in socially disadvantageous groups which was previously reported for non-whites [6]. However, our data from a European cohort, for the first time, extends the concept of a social gradient in left ventricular mass to Caucasians and to systolic cardiac function. Rodriguez et al. reported a higher left ventricular mass in socially isolated subjects, which might be one explanation for the results of our study [5]. Social isolation might also be one trigger for the social gradient of obesity that was mainly observed in females. This supports our finding of a stronger explanatory power of BMI for educational differences in ventricular mass and ejection fraction among female subjects.

Limitations

Our analyses measured the ejection fraction by means of the Teichholz formula in M-mode, which is, however, not recommended for clinical use in current guidelines (biplane-based methods preferred). However, the sensitivity analysis showed that results and inferences are unlikely to differ substantially if this method was used. Despite this limitation, we do not expect it to be a major source of biased effect estimates as an increased non-differential measurement error, which is likely due to an augmented inaccuracy and thus diversification of measurements. However, this would lead to wider confidence intervals than expected with more advanced echocardiographic methods. In the light of this consideration, we expect future studies using more advanced methods for the measurement of the ejection fraction to find even stronger relations and to disclose statistical evidence despite weak mediation effects due to a higher validity and precision. This might explain why we failed to disclose a statistically more convincing (equivalent to a smaller error term in the model) mediation effect of BMI on ejection fraction.

Furthermore, our results apply to a collective with an age range of 45–87 years, thus relations might be different in younger or, more importantly, even older subjects.

Finally, although it is unlikely that the general conclusion might change due to unobserved confounding factors, effect estimates might change nevertheless.

Conclusion

In conclusion, we found temporally (during a four-year period) constant differences in ventricular mass and ejection fraction in female subjects with different levels of education. More importantly, the mediation analyses revealed that obesity (defined by means of the BMI) is the key mediator of these disparities contributable to educational levels accounting for more than 50% of the differences in ventricular mass and roughly 30% of the differences in EF.

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Inflammation and echocardiographic parameters of ventricular hypertrophy in a cohort with preserved cardiac function

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ABSTRACT

Objective: To investigate the association between inflammation and selective echocardiographic parameters (EP) characteristic for ventricular hypertrophy in cross-sectional and longitudinal population-based analyses.

Methods: Baseline (711 men, 659 women: 45–83 years) and 4-year follow-up data (622 men, 540 women) of the prospective, population-based CARdiovascular disease, Living and Ageing in Halle (CARLA) study after exclusion of participants with cardiacvascular diseases were analysed. Inflammation parameters: soluble tumour necrosis factor receptor 1 (sTNF-R1), high-sensitivity C reactive protein (hsCRP) and interleukin 6 (IL-6). EPs: left ventricular mass (LVM), left atrial systolic dimension (LADS), interventricular septum diameter (IVSD), posterior wall dimension (PWD), left ventricular diastolic diameter (LVDD), ejection fraction according to Teichholz (EF). For the longitudinal analyses baseline to follow-up differences were considered. Effect sizes were determined by using multiple linear regression and mixed models. Missing values were replaced by means of multiple imputations.

Results: Men had higher sTNF-R1 levels; means of hsCRP and IL-6 were similar in men and women. In multiple regression models, sTNF-R1 was associated with LADS (1.4 mm/1000 pg/mL sTNF-R1, 95% CI 0.6 to 2.1) in men. Respecting confounder hsCRP was associated with LVM (5.2 g/10 mg/L hsCRP, 95% CI 1.6 to 8.8), IVSD (0.2 mm/10 mg/L hsCRP, 95% CI 0 to 0.3) and PWD (0.2 mm/10 mg/L hsCRP, 95% CI 0.1 to 0.3) in women, while there were no relevant effects in analysis of IL-6 in both sexes. The baseline to follow-up change in EPs was not relevantly associated with sTNF-R1, hsCRP or IL-6.

Conclusions: STNF-R1, hsCRP and IL-6 were inadequate predictors for structural changes of the heart at follow-up, while weak cross-sectional associations are restricted to certain EPs and depend on sex.

INTRODUCTION

The prognostic role of cytokines and their corresponding modulators, especially soluble tumour necrosis factor receptor 1 (sTNF-R1)[1 2] and C reactive protein (CRP),[3–5] in the development of lethal outcomes of congestive systolic and diastolic heart failure (CHF) has been revealed previously. The value of cytokines in predicting death in patients with myocardial infarction was the subject of further studies.[6 7] They found that higher levels of sTNF-R1, but not interleukin 6 (IL-6) and CRP, were associated with an increased risk of death. This is in contrast to the findings reported by Tan *et al*[8] who showed that IL-6 was an independent predictor of cardiovascular mortality. Further cross-sectional studies focused on the relationship between IL-6, sTNF-R1 and CRP, respectively, and left ventricular hypertrophy (LVH) in the general population[9] and in asymptomatic hypertensive patients.[10] The authors revealed a positive relationship between sTNF-R1 and LVH, but failed to find a similar effect for IL-6 and CRP, which was associated with LVH in another collective of asymptomatic participants with essential hypertension.[11] Although there is evidence from experimental studies indicating the pathophysiological role of IL-6 in the development of cardiac hypertrophy[12] the impact of IL-6 on cardiac hypertrophy in humans remains controversial.

Summarising previous findings, sTNF-R1 was suggested to be of prognostic value for the course of disease in cardiac patients. The stability of sTNF-R1 makes it an easily assessable marker of the larger TNF system.[13] Interestingly, the type 1 receptor of TNF-α is the origin of several pathways in the human heart, affecting cell metabolism, apoptosis and remodelling.[2] The soluble form of this receptor is released from its membrane-bound component by different stimuli (eg, TNF-α, lipopolysaccharides), and serves among other things as a ligand to

TNF-α.[14 15] The shedding of sTNF-R1 is increased in patients with heart failure,[16] myocardial infarction[17] and coronary artery disease.[18]

Although the association between sTNF-R1 and CHF or LVH, respectively, has been examined previously in clinical populations, there is a substantial lack of studies examining the longitudinal association of inflammation with LVH assessed via echocardiographic parameters of ventricular wall thickness, dimensions and ejection fraction (EF) in cardiovascularly healthy participants. Therefore, the objectives of the current study were

1. To analyse the cross-sectional association of inflammation—especially sTNF-R1—with echocardiographic parameters related to ventricular hypertrophy in a cardiovascularly healthy population-based cohort, avoiding possible distractions due to pre-existing cardiac defects;
2. To assess the prognostic value of inflammation for changes in the parameters aforementioned in a 4-year period as a longitudinal analyses in this group of participants.

METHODS

Study design and study population

We used data from the *CARdio-vascular disease, Living and Ageing in Halle* (CARLA) study, which is a prospective population-based cohort study of the elderly general population of the city of Halle in eastern Germany.[19] The CARLA cohort comprises 1779 participants (baseline response 64.1%), aged 45–83 years at baseline (967 men and 812 women). The baseline examination took place between December 2002 and January 2006. A multistep recruitment strategy aimed to achieve a high response rate. The percentage final response after subtracting exclusions (individuals who were deceased prior to the invitation, had moved away, or were unable to participate due to illness) was 64%. From March 2007 until March 2010, the first 4-year follow-up examination was performed. The net sample (after exclusion of deceased or non-responding people) then comprised 1436 participants (86% response), consisting of 790 men and 646 women aged between 50 and 87 years. The study participants underwent a detailed medical examination and a standardised, computer-assisted interview, which collected information on sociodemographic and socioeconomic variables, behavioural, biomedical and psychosocial factors, medical history and the use of medication within the preceding 7 days. Medication was automatically coded according to the Anatomical Therapeutic Chemical Classification System (ATC code). Additionally, an analysis of non-respondents was performed in order to assess non-response bias by obtaining information about prevalent diseases, and selected behavioural and sociodemographic factors. A more comprehensive account of the CARLA study can be found in Greiser *et al*.[19 20] All participants gave written informed consent. We included all patients without clinical and echocardiographic signs of CHF including elevated probrain natriuretic peptide. A more comprehensive account of the definition of CHF in the CARLA study can be found elsewhere.[21] In short, CHF was defined as follows: presence of symptoms of CHF (oedema, fatigue and dyspnoea) and an NT-probrain natriuretic peptide (NT-proBNP) above 220 pg/mL or a reduced EF (<50% according to the Teichholz *et al*'s[22] formula), or a left ventricular (LV) dimension index above 3.8 cm/m^2 in men and 3.7 cm/m^2 in women and echocardiographic parameters of diastolic dysfunction, which is in accordance with international guidelines.[23] Additionally, we excluded all participants with a history of myocardial infarction and presence of cardiovascular diseases (history of stroke, history of vascular intervention—self-reported and subsequently physician approved). Thus, the final study sample comprised 711 men (256 excluded) and 659 women (153 excluded) at baseline and 622 men and 540 women at follow-up, while 52 men and 33 women primarily included in the study died between baseline and follow-up.

Echocardiographic assessment

At baseline, Doppler echocardiographic examinations were conducted and evaluated by a specially trained and certified physician. At follow-up, echocardiography was performed by a trained and certified study nurse, and subsequently the stored echocardiographic recordings were evaluated by a trained physician. All echocardiographers underwent the same dedicated study certification procedures. Assessing intraobserver variability for the M-mode examinations the mean observer bias varied between 0.3% and 3.8% (2*SD between 15.3% and 27.7%), while the interobserver variability ranged between 0.1% and 2.7% (2*SD between 12.7% and 20.8%). All echocardiographic examinations at baseline and follow-up were performed using the GE Vivid ultrasound system (GE Vivid 4 and 5 at baseline, GE vivid 5 at follow-up). To quantify the LV dimensions and function, we chose echocardiographic parameters (table 1) that are recommended by the guidelines for chamber quantification in echocardiography.[24] We took only dimensional parameters, rather than volume parameters, into account. To calculate the left ventricular mass (LVM), we used the ASE-cube formula, which is in accordance with international guidelines.[24] In addition, the size of the left ventricle was quantified according to the LV diastolic dimension (LVDD). The LV wall thickness was assessed by measuring the diastolic interventricular septum thickness (IVSD) and the diastolic thickness of the posterior wall (PWD). In order to examine the cross-sectional association of inflammation with the myocardial geometry more closely, we differentiated between participants with signs of concentric remodelling and participants without such changes. According to Lang *et al*[24] concentric remodelling was defined based on the relative posterior wall thickness (RPWD), which was calculated by the formula RPWD=

Table 1 Baseline characteristics

	Baseline				Follow-up			
	Men		Women		Men		Women	
Miscellaneous	N	Mean (95% CI)	N	Mean (95% CI)	N	Mean (95% CI)	N	Mean (95% CI)
Age (years)	711	62 (61 to 63)	659	62 (61 to 63)	621	66 (65 to 66)	540	65 [64 to 66]
BSA (m^2)	711	1.98 (1.98 to 1.98)	659	1.75 (1.74 to 1.77)	618	1.98 (1.96 to 1.99)	534	1.75 [1.73 to 1.76]
AMP (mm Hg)	711	106.57 (105.67 to 107.47)	659	102.01 (101.02 to 103.01)	619	100.06 (99.12 to 101.01)	535	97.3 [96.32 to 98.28]
BMI (kg/m^2)	711	27.67 (27.47 to 27.87)	659	27.76 (27.5 to 28.01)	618	27.79 (27.57 to 28.02)	534	27.74 [27.44 to 28.06]
Echocardiographic parameters								
LV mass (g)*	691	228.64 (224.49 to 232.86)	460	220.77 (215.99 to 225.65)	460	220.77 (215.99 to 225.65)	425	166.34 [162.71 to 170.05]
PWD (mm)*	692	11.9 (11.79 to 12.02)	463	11.1 (10.92 to 11.28)	463	11.1 (10.92 to 11.28)	425	10 [9.82 to 10.18]
IVSD (mm)*	695	11.87 (11.75 to 12)	462	11.63 (11.44 to 11.81)	462	11.63 (11.44 to 11.81)	428	10.57 [10.4 to 10.74]
LAD (mm)*	683	40.08 (39.73 to 40.43)	579	40.92 (40.53 to 41.31)	579	40.92 (40.53 to 41.31)	506	37.81 [37.41 to 38.21]
LVDd (mm)*	693	49.39 (48.88 to 49.9)	464	50.17 (49.66 to 50.68)	464	50.17 (49.66 to 50.68)	426	45.85 [45.36 to 46.35]
EF (%)	690	62.78 (62.16 to 63.4)	458	65.54 (64.67 to 66.43)	458	65.54 (64.67 to 66.43)	422	67.93 [67.03 to 68.84]
Blood parameters								
TNF-R1 (pg/mL)	672	1122.62 (1093.02 to 1153.03)	614	1021.97 (994.12 to 1050.6)				
hsCRP (mg/L)	681	1.67 (1.54 to 1.8)	642	1.85 (1.7 to 2.01)	619	1.84 (1.71 to 1.99)	531	1.85 [1.7 to 2.01]
IL-6 (pg/mL)	672	1.99 (1.83 to 2.17)	614	1.8 (1.65 to 1.97)				
Creatinine (µmol/L)	708	78.41 (77.33 to 79.52)	659	63.34 (62.48 to 64.22)	619	82.37 (80.82 to 83.96)	531	66.74 [65.66 to 67.83]
HDL (mmol/l)	690	3.11 (3.04 to 3.17)	656	3.32 (3.25 to 3.39)	606	3.05 (2.98 to 3.12)	529	3.36 [3.28 to 3.44]
HbA1c (%)	708	5.67 (5.61 to 5.73)	659	5.64 (5.59 to 5.7)	619	5.79 (5.73 to 5.84)	531	5.79 [5.73 to 5.84]
Numeric values								
Median n of medication	711	4	659	4	619	4	535	4

Means are displayed as geometric means with the respecting 95% confidence limits.
*Adjusted for body surface area.
AMP, Arterial mean pressure; BMI, body mass index; BSA, Body surface area; CRP, high-sensitivity C reactive protein; EF, left ventricular ejection fraction; HDL, high-density lipoproteinhs; IL, interleukin 6; IVSD, interventricle septum diameter; LAD, left atrial diastolic diameter; LV, left ventricular mass; LVDd, left ventricular end-diastolic dimension; PWD, posterior wall dimension; TNF, soluble tumour necrosis factor-α receptor 1.

(2×PWD)/LVDD.[24] Finally, an RPWD above 0.42 was considered a concentrically altered LV. The cardiac output as a functional parameter was measured by the EF according to Teichholz *et al*.[22] We added the systolic dimension of the left atrium (LADS) to our analysis, as this parameter was previously found to be related to cardiac risk.[24]

Laboratory measurements

Blood samples were taken after a supine rest of 30 min at baseline and at follow-up. At baseline the parameters of sTNF-R1, high-sensitivity CRP (hsCRP) and IL-6 were assessed, while hsCRP was measured at follow-up as well.

STNF-R1 and IL-6 were analysed by the Department of Medicine III, University Clinics Halle (Saale). After 10 min centrifugation (20°C, 1500 rpm, Acc=9, Dcc=3), the plasma was collected and stored at –80°C. Cytokines were assessed using commercially available sandwich ELISAs (IL-6, Opteia, BD Biosciences, Heidelberg, Germany; TNF-R1, Boehringer Mannheim, Mannheim, Germany).

The determination of hsCRP was undertaken by the Institute of Laboratory Medicine, Clinical Chemistry and Molecular Diagnostics at the Leipzig University Clinics. The laboratory has been accredited according to the accreditation norms ISO 15180 and ISO 17025. Serum hsCRP levels were measured using a high-sensitivity immunoturbidimetric method (CRP (Latex) HS, Roche, Mannheim, Germany) on a Hitachi autoanalyser (Roche Diagnostics Mannheim, Germany).

Statistical methods

All analyses were performed separately for men and women. Descriptive results are displayed as geometric means to illustrate the distribution of echocardiographic and inflammation parameters in the study population.

To assess the association of sTNF-R1, hsCRP and IL-6 (each as an independent variable) with all six echocardiographic parameters in a multivariate approach we used mixed models with an unstructured covariance matrix respecting echocardiographic parameters as a fixed effect. These analyses were separately conducted for sTNF-R1, hsCRP and IL-6, men and women, and the cross-sectional and longitudinal analyses, respectively (statistical results given in tables 2–4). Parameters were entered in the mixed model after standardisation of all included dependent and independent parameters (mean=0, SD=1) making the association across all echocardiographic parameters comparable between analyses. In addition, single regression coefficients and respective 95% CIs (association of each inflammation parameter with each echocardiographic parameter) were calculated by means of univariate linear robust regression models. Models were adjusted for arterial mean pressure, body surface area, age, medication, glycated haemoglobin (HbA1c), low-density lipoprotein and creatinine clearance. As medication, we considered antihypertensive drugs (ATC codes: C02/C03/C07/C08/C09), cardiac glycosides (ATC code: C01A) and lipid-lowering medication (ATC code: C10). In the longitudinal regression analysis, we used the difference in echocardiographic values between baseline and follow-up as outcome without consideration of baseline echocardiographic values as a further covariate.[25] The idea beyond such a longitudinal analysis is to check for associations of a change in one parameter over time (from baseline to follow-up), depending on an explanatory variable, which influences the course of the dependent parameter. Possible differences in the blood level of inflammatory parameters between participants with signs of a concentrically altered LV wall thickness compared with participants without such signs were checked by performing a Mann-Whitney U test. Echocardiographic parameters adjusted for body surface area are specified in the descriptive statistics. Finally, the adequacy of the considered regression models was assumed when the residuals were normally distributed, which was tested via a Q-Q plot and Cook's distance, which was required to be below one (achieved for all conducted tests).

Missing values were replaced using the method of multiple imputation, which was previously found to cause little bias[26] (see online supplementary appendix for further details). Deviations of the results between multiple imputations and the complete case analysis were compared in a sensitivity analysis, which is given in the appendix. A further sensitivity analysis was performed after exclusion of participant with the presence of self-reported and physician evaluated chronic diseases related to inflammation (cancer, rheumatoid arthritis, gout, liver disease, chronic kidney disease with a glomerular filtration rate below 30 mL/min).

The level of significance was taken as α=0.05, and consequently we report the 95% confidence limits. All statistical analyses and data management were performed using SAS, V.9.3 (SAS Inc, Cary, North Carolina, USA).

RESULTS

Study population

In the final sample, the mean age at baseline of male and female participants was 62 years (95% CI 61 to 63; table 1). We observed a higher EF but lower echocardiographic dimension and LV wall thickness parameters in women compared to men. While men showed a higher concentration of plasma sTNF-R1 (table 1), no considerable differences in plasma levels of hsCRP and IL-6 between sexes could be observed. At follow-up, our data indicated a decrease in LVM and an increase in EF, while there were no relevant changes in the mean of further echocardiographic parameters.

Cross-sectional association of inflammation and echocardiographic parameters

Soluble tumour necrosis factor receptor 1

Most of the participants had blood levels of sTNF-R1 between 500 and 2000 pg/mL, although 5.65% of men

Table 2 Estimates of the regression analysis of sTNF-R1

	Men				Women			
	Core		Adjusted		Core		Adjusted	
sTNF-R1	β (95% CI)	p Value	β (95% CI)	p Value	β (95% CI)	p Value	β (95% CI)	p Value
Cross sectional*	0.11 (0.07 to 0.14)	<0.0001	0.06 (0.02 to 0.1)	0.0038	0.15 (0.1 to 0.2)	<0.0001	0.04 (−0.01 to 0.09)	0.0998
LVM (g)	13.8 (5.6 to 21.9)	0.0011	5.7 (−2.7 to 14)	0.1820	22.8 (14 to 31.6)	<0.0001	3.5 (−4.5 to 11.5)	0.3932
PWD (mm)	0.6 (0.4 to 0.8)	<0.0001	0.3 (0 to 0.5)	0.0231	1 (0.8 to 1.3)	<0.0001	0.3 (0.1 to 0.6)	0.0121
IVSD (mm)	0.6 (0.3 to 0.8)	<0.0001	0.2 (−0.1 to 0.4)	0.1614	1 (0.7 to 1.3)	<0.0001	0.3 (0 to 0.6)	0.0267
LADS (mm)	2.1 (1.3 to 2.8)	<0.0001	1.4 (0.6 to 2.1)	0.0010	2.5 (1.7 to 3.4)	<0.0001	0 (−0.9 to 0.8)	0.9755
LVDD (mm)	0 (−0.8 to 0.8)	0.9796	0.2 (−0.7 to 1)	0.6835	0 (−0.9 to 0.9)	0.9925	−0.7 (−1.6 to 0.2)	0.1532
EF (%)	−0.3 (−1.4 to 0.9)	0.6442	−0.1 (−1.4 to 1.2)	0.8885	0.5 (−0.8 to 1.7)	0.4666	0.6 (−0.9 to 2.1)	0.4003
Longitudinal*	−0.44 (−0.92 to 0.03)	0.0675	−0.02 (−0.08 to 0.04)	0.4641	0.18 (−0.34 to 0.7)	0.4873	0.44 (−0.14 to 1.02)	0.1320
LVM (g)	−8.1 (−19.3 to 3.1)	0.1568	−3.3 (−16.4 to 9.8)	0.6181	−1 (−11 to 9.1)	0.8455	5.8 (−6.1 to 17.7)	0.3361
PWD (mm)	−0.4 (−0.8 to 0.1)	0.1031	−0.2 (−0.7 to 0.3)	0.4765	−0.3 (−0.8 to 0.2)	0.3043	−0.1 (−0.7 to 0.5)	0.6642
IVSD (mm)	−0.1 (−0.6 to 0.3)	0.6007	0.1 (−0.4 to 0.7)	0.5619	−0.3 (−0.8 to 0.2)	0.2469	0 (−0.6 to 0.6)	0.9177
LADS (mm)	0.1 (−0.8 to 1)	0.8342	0.1 (−0.9 to 1.1)	0.8406	0.6 (−0.3 to 1.5)	0.2162	0.7 (−0.4 to 1.7)	0.2073
LVDD (mm)	−0.2 (−1.4 to 1.1)	0.7971	−0.3 (−1.7 to 1.1)	0.6880	1.5 (0.1 to 2.8)	0.0340	1.7 (0.1 to 3.3)	0.0357
EF (%)	−1.3 (−3.4 to 0.7)	0.2084	−1.7 (−4.1 to 0.6)	0.1501	−0.5 (−3.1 to 2)	0.6742	−1 (−3.9 to 1.9)	0.4931

Estimates of the longitudinal analyses mean the absolute baseline to follow-up difference of echocardiographic parameters.
Adjusted for arterial mean pressure, body surface area, age, medication, HbA1c, LDL and creatinine clearance.
*(grey-shaded line)=results from mixed models estimating the association of inflammation parameter across all six echocardiographic parameters in the cross-sectional, and longitudinal analyses, respectively. Estimates were standardised (mean=0, SD=1).
EF, ejection fraction; HbA1c, glycated haemoglobin; IL-6, interleukin 6; IVSD, interventricle septum diameter; LADS, left atrial diastolic diameter; LVDD, left ventricular end-diastolic dimension; LDL, low-density lipoprotein; LVM, left ventricular mass; PWD, posterior wall dimension; sTNF-R1, soluble tumour necrosis factor-α receptor 1.

Table 3 Estimates of the regression analysis of hsCRP

	Men				Women			
	Core		Adjusted		Core		Adjusted	
hsCRP	β (95% CI)	p Value	β (95% CI)	p Value	β (95% CI)	p Value	β (95% CI)	p Value
Cross sectional*	0.02 (−0.03 to 0.06)	0.5024	0.02 (−0.03 to 0.06)	0.4821	0.04 (0.02 to 0.07)	0.0004	0.03 (0.01 to 0.05)	0.0133
LVM (g)	1 (−8.7 to 10.6)	0.8442	−1.9 (−10.2 to 6.3)	0.6449	12.7 (3.6 to 21.8)	0.0064	5.2 (1.6 to 8.8)	0.0051
PWD (mm)	0.2 (0 to 0.5)	0.0800	0.1 (−0.2 to 0.3)	0.5006	0.5 (0.2 to 0.9)	0.0012	0.2 (0.1 to 0.3)	0.0013
IVSD (mm)	0.2 (−0.1 to 0.5)	0.1788	0.1 (−0.2 to 0.3)	0.5734	0.5 (0.2 to 0.8)	0.0016	0.2 (0 to 0.3)	0.0072
LADS (mm)	0.7 (−0.2 to 1.7)	0.1156	0.4 (−0.4 to 1.3)	0.3073	0.8 (0.1 to 1.5)	0.0337	0.2 (−0.2 to 0.6)	0.2310
LVDD (mm)	−0.2 (−1.1 to 0.7)	0.6415	−0.4 (−1.3 to 0.5)	0.3904	0.1 (−0.4 to 0.7)	0.5882	−0.2 (−0.6 to 0.3)	0.4811
EF (%)	−0.3 (−1.6 to 1.1)	0.6807	−0.3 (−1.6 to 1.1)	0.6846	−0.3 (−1.1 to 0.6)	0.5447	−0.3 (−1 to 0.4)	0.4336
Longitudinal*	−0.01 (−0.56 to 0.55)	0.9851	−0.01 (−0.06 to 0.05)	0.9289	−0.12 (−0.75 to 0.52)	0.7102	−0.01 (−0.07 to 0.06)	0.8031
LVM (g)	3.2 (−9.1 to 15.6)	0.6043	3.4 (−9.4 to 16.2)	0.5954	−3 (−13.8 to 7.9)	0.5888	−1.4 (−12.5 to 9.7)	0.8064
PWD (mm)	−0.2 (−0.7 to 0.3)	0.4190	−0.2 (−0.6 to 0.3)	0.4952	−0.3 (−0.9 to 0.2)	0.2418	−0.2 (−0.8 to 0.3)	0.4197
IVSD (mm)	0.1 (−0.4 to 0.5)	0.8090	0.1 (−0.4 to 0.5)	0.8327	−0.2 (−0.7 to 0.3)	0.4724	−0.1 (−0.6 to 0.4)	0.7357
LADS (mm)	0.8 (−0.2 to 1.8)	0.1149	0.6 (−0.3 to 1.6)	0.1928	1.1 (0.1 to 2.1)	0.0376	0.9 (−0.1 to 2)	0.0878
LVDD (mm)	0.7 (−0.6 to 2)	0.3191	0.6 (−0.7 to 2)	0.3687	0.5 (−1.1 to 2)	0.5424	0.4 (−1.2 to 2)	0.6083
EF (%)	−1.5 (−3.5 to 0.6)	0.1559	−1.5 (−3.6 to 0.6)	0.1491	−0.3 (−3 to 2.4)	0.8181	−0.2 (−2.9 to 2.6)	0.9117

Estimates of the longitudinal analyses mean the absolute baseline to follow-up difference of echocardiographic parameters.
Adjusted for arterial mean pressure, body surface area, age, medication, HbA1c, LDL and creatinine clearance.
*(grey shaded line)=results from mixed models estimating the association of inflammation parameter across all six echocardiographic parameters in the cross-sectional, and longitudinal analyses, respectively. Estimates were standardised (mean=0, SD=1).
hsCRP, high-sensitivity C reactive protein, EF, ejection fraction; IL-6, interleukin 6; IVSD, interventricle septum diameter; LADS, left atrial diastolic diameter; LVDD, left ventricular end-diastolic dimension; LDL, low-density lipoprotein; LVM, left ventricular mass; PWD, posterior wall dimension.

Table 4 Estimates of the regression analysis of IL-6

	Men				Women			
	Core		Adjusted		Core		Adjusted	
IL-6	β (95% CI)	p Value	β (95% CI)	p Value	β (95% CI)	p Value	β (95% CI)	p Value
Cross sectional*	−0 (−0.05 to 0.05)	0.9471	−0.01 (−0.05 to 0.04)	0.7432	0.08 (−0.02 to 0.17)	0.1031	0.07 (−0.01 to 0.14)	0.0834
LVM (g)	2.1 (−2.3 to 6.5)	0.3321	−0.3 (−1.9 to 1.2)	0.6782	2 (−1.5 to 5.5)	0.2516	0.3 (−3 to 3.7)	0.8377
PWD (mm)	0.1 (0 to 0.2)	0.0761	0.1 (0 to 0.2)	0.0794	0.1 (0 to 0.3)	0.0956	0.1 (0 to 0.2)	0.1962
IVSD (mm)	0.1 (0 to 0.2)	0.1178	0 (−0.1 to 0.2)	0.5380	0.1 (−0.1 to 0.2)	0.2309	0.1 (0 to 0.1)	0.1552
LADS (mm)	0.3 (−0.1 to 0.6)	0.0984	0.1 (−0.1 to 0.3)	0.5914	0.2 (−0.3 to 0.6)	0.4621	0.2 (−0.2 to 0.5)	0.2764
LVDD (mm)	−0.1 (−0.5 to 0.3)	0.4617	−0.1 (−0.5 to 0.3)	0.7001	−0.1 (−0.3 to 0.2)	0.6394	−0.1 (−0.4 to 0.1)	0.2281
EF (%)	−0.1 (−0.6 to 0.3)	0.5474	−0.3 (−0.5 to 0)	0.0468	0 (−0.7 to 0.8)	0.9634	0.1 (−0.4 to 0.7)	0.6011
Longitudinal*	0.05 (−0.46 to 0.55)	0.8498	0.01 (−0.04 to 0.06)	0.7329	−0.67 (−1.44 to 0.11)	0.0915	−0.06 (−0.14 to 0.01)	0.1070
LVM (g)	−3.2 (−8.3 to 1.9)	0.2067	−1.7 (−7.4 to 4)	0.5384	−0.1 (−4.8 to 4.5)	0.9562	−0.9 (−3.7 to 1.9)	0.5319
PWD (mm)	−0.2 (−0.3 to 0)	0.1101	−0.1 (−0.4 to 0.1)	0.2244	−0.1 (−0.2 to 0.1)	0.2689	−0.1 (−0.2 to 0.1)	0.2506
IVSD (mm)	0 (−0.2 to 0.2)	0.8388	0.1 (−0.1 to 0.2)	0.3519	0 (−0.3 to 0.2)	0.6943	−0.1 (−0.2 to 0.1)	0.3989
LADS (mm)	0 (−0.4 to 0.3)	0.7980	0 (−0.1 to 0.2)	0.6791	−0.1 (−0.7 to 0.4)	0.6510	−0.4 (−0.7 to 0)	0.0656
LVDD (mm)	−0.1 (−0.3 to 0.2)	0.5102	−0.1 (−0.3 to 0.1)	0.4871	0.1 (−0.2 to 0.5)	0.4453	0.1 (l−0.2 to 0.5)	0.5017
EF (%)	0 (−0.8 to 0.8)	0.9729	0.2 (−0.6 to 1)	0.6496	0 (−0.7 to 0.7)	0.9795	0 (−0.6 to 0.7)	0.9485

Estimates of the longitudinal analyses mean the absolute baseline to follow-up difference of echocardiographic parameters.
Adjusted for arterial mean pressure, body surface area, age, medication, HbA1c, LDL and creatinine clearance.
*(grey-shaded line)=results from mixed models estimating the association of inflammation parameter across all six echocardiographic parameters in the cross-sectional, and longitudinal analyses, respectively. Estimates were standardised (mean=0, SD=1).
EF, ejection fraction; HbA1c, glycated haemoglobin; IL-6, interleukin 6; IVSD, interventricle septum diameter; LADS, left atrial diastolic diameter; LVDD, left ventricular end-diastolic dimension; LDL, low-density lipoprotein; LVM, left ventricular mass; PWD, posterior wall dimension.

and 3.09% of women showed even higher values (see online supplementary figure A1). The estimates obtained from mixed models (covariate adjusted) respecting all six echocardiographic parameters indicated an overall association of sTNF-R1 with echocardiography in men, but not in women. The univariate analysis of echocardiographic values as a function of inflammatory parameters revealed a relevant unadjusted association of sTNF-R1 with LVM, IVSD, PWD and LADS (table 2). Nevertheless, after the models were adjusted for the considered covariates, the regression coefficients decreased considerably. A medium effect size remained only when LADS (1.4 mm/1000 pg/mL sTNF-R1, 95% CI 0.6 to 2.1) was the outcome in men, while the association of sTNF-R1 with PWD is of minor clinical relevance. The regression analysis of EF as a functional parameter revealed no relevant association with sTNF-R1 (see online supplementary figure A2). Consistent with the findings of the regression analysis of LVDD and PWD, the differentiation between concentric remodelling and ordinary/eccentric myocardial tissue (figures 1 and 2, see the online supplementary appendix for the results across both sexes) indicated that men and women with signs of concentric alterations had considerably higher blood levels of sTNF-R1.

High-sensitivity C reactive protein

The majority of the study population had plasma hsCRP levels below 10 mg/L (see online supplementary figure A1). A general association of hsCRP with echocardiographic parameters could only be found in women, which was mainly due to low statistical variance (0.03, 95% CI 0.01 to 0.05), while the estimate itself was lower than in the case of sTNF-R1. The multivariate analysis revealed lower effect estimates in men than in women. When covariates were taken into account, hsCRP was positively associated with LVM (5.2 g/10 mg/L hsCRP, 95% CI 1.6 to 8.8), PWD (0.2 mm/10 mg/L hsCRP, 95% CI 0.1 to 0.3) and IVSD (0.2 mm/10 mg/L hsCRP, 95% CI 0 to 0.3) in female participants, though the estimates

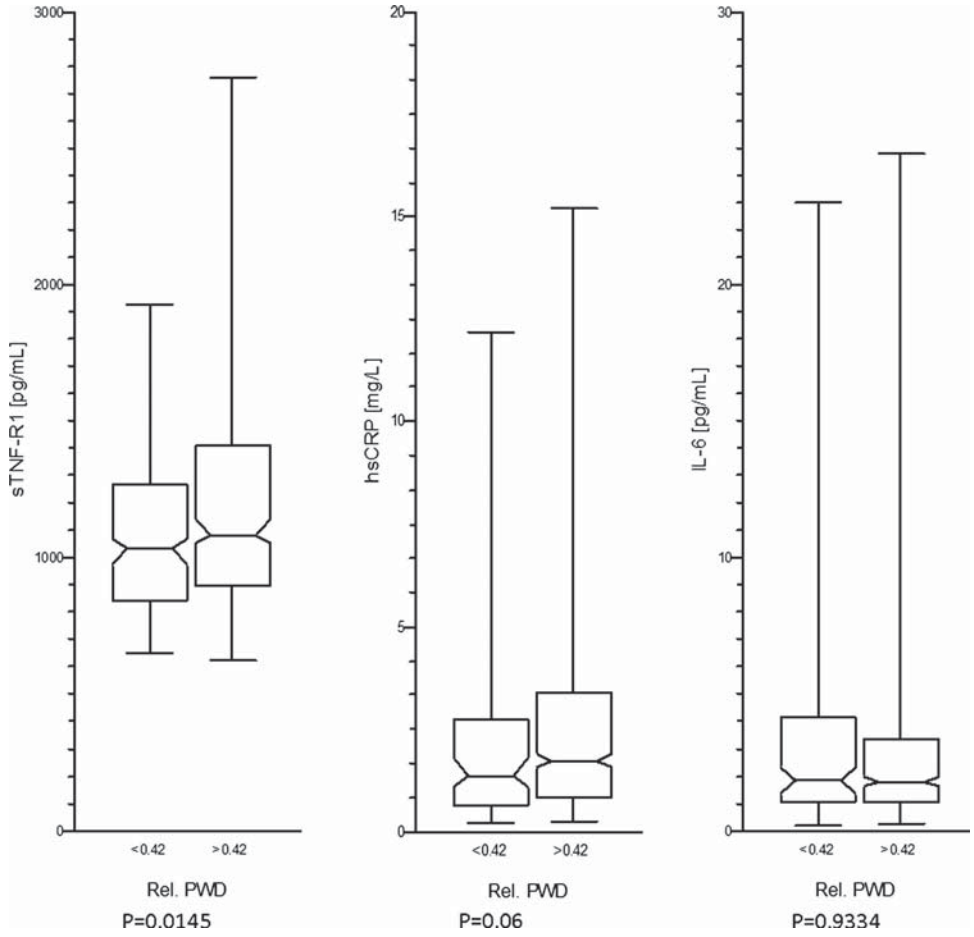

Figure 1 Box plot comparing inflammation parameters in men with (RPWD<0.42) and without (RPWD>0.42) signs of concentric hypertrophy. Lower and upper whiskers display the 2% and 98% quintiles, respectively. Notches display the 95% CI of the median (shortened horizontal line). The box represents participants between 25% and 75% quintiles. p Values refer to group comparison (RPWD<0.42 and RPWD>0.42) by means of a Mann-Whitney U test. hsCRP, high-sensitivity C reactive protein; IL-6, interleukin 6; RPWD, relative posterior wall dimension of the left ventricle; sTNF-R1, soluble tumour necrosis factor-α receptor 1.

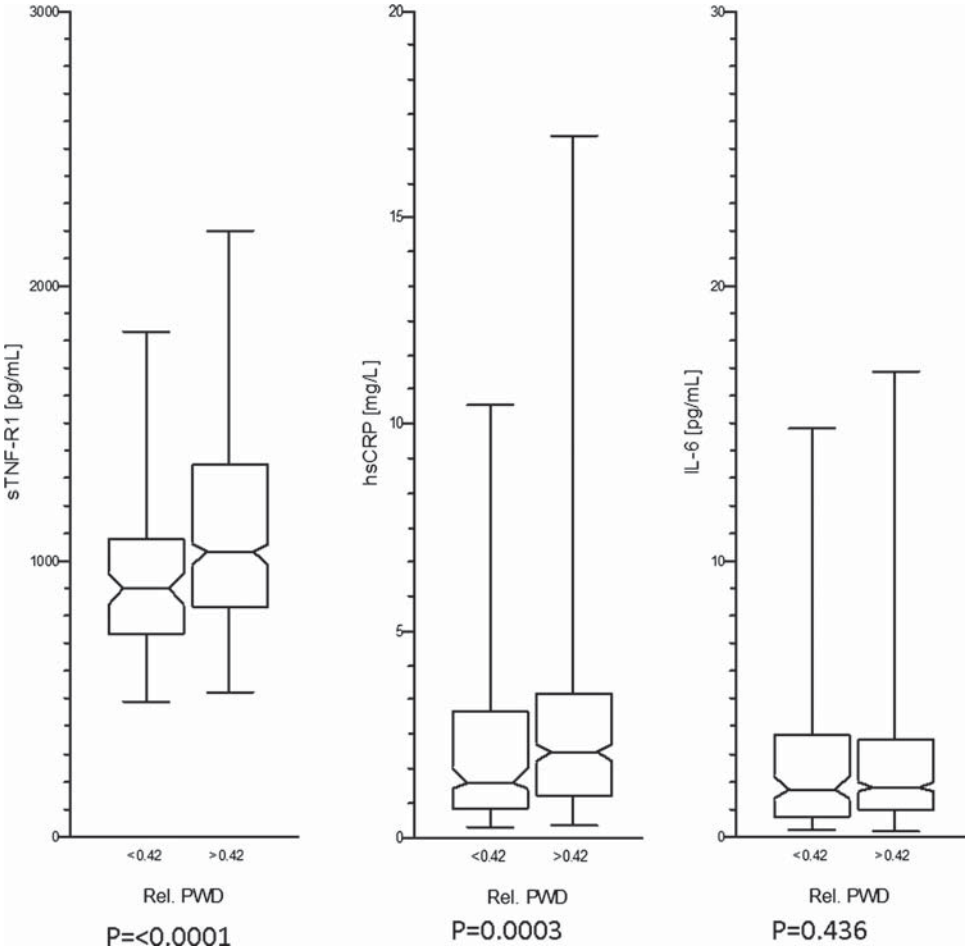

Figure 2 Box plot comparing inflammation parameters in women with (RPWD<0.42) and without (RPWD>0.42) signs of concentric hypertrophy. Lower and upper whiskers display the 2% and 98% quintiles, respectively. Notches display the 95% CI of the median (shortened horizontal line). The box represents participants between 25% and 75% quintiles. p Values refer to group comparison (RPWD<0.42 and RPWD>0.42) by means of a Mann-Whitney U test. hsCRP, high-sensitivity C reactive protein; IL-6, interleukin 6; RPWD, relative posterior wall dimension of the left ventricle; sTNF-R1, soluble tumour necrosis factor-α receptor 1.

appeared distinctly lower than in the unadjusted analyses (table 3). However, the effects were weak, which is especially obvious when the lower confidence limit is considered. Again, participants with signs of concentric remodelling had a higher CRP level in both sexes (figures 1 and 2).

Interleukin 6

Distributions of IL-6 and hsCRP showed a similar pattern with mainly low blood levels (see online supplementary figure A1). According to the large CIs of the regression analysis, the IL-6 levels were not related to the considered structural parameters (table 4) in men and women. After covariate adjustment the estimates declined, leading to negligible effect sizes. In the same line, IL-6 was not notably associated with the functional parameter of EF in men or women. Participants with echocardiographic signs of concentric hypertrophy and without such indications showed almost identical IL-6 blood levels (figures 1 and 2).

Longitudinal association of inflammation and echocardiographic parameters

In the longitudinal analysis, we found a slight association of sTNF-R1 and LVDD in women in the univariate (1.5 mm/1000 pg/mL, 95% CI 0.1 to 2.8) and multivariate models (1.7 mm/1000 pg/mL, 95% CI 0.1–3.3), which was much lower in men. However, this finding might be due to multiple testing, as there was no overall association of sTNF-R1 with the change of echocardiographic parameters when the results of the mixed models are taken into account. All other regression analyses of change in echocardiographic parameters depending on sTNF-R1, IL-6 and hsCRP revealed only minimal, clinically negligible associations (tables 2–4).

Sensitivity analyses

The performance of a complete case analysis led to higher effect estimates and significant results in few instances (see online supplementary tables A1–3), which might be mainly due to bias driven by missing values

and outliers. The exclusion of participants with chronic diseases associated with inflammation was not related to relevant alterations of effect estimates (see online supplementary tables A4–6).

DISCUSSION AND LIMITATIONS

We found minor associations of plasma sTNF-R1 with PWD and LADS in men, and IVSD in women, while plasma hsCRP was slightly associated with LVM, PWD and IVSD in female participants. In the longitudinal analyses no relevant associations could be deduced from our data. In summary this reflects only a weak association of systemic inflammation as measured by plasma levels with structural echocardiographic parameters of ventricular hypertrophy and atrial size.[27] However, LVM and the size of the left atrium were both reported as being independently associated with hospitalisation and death, respectively.[28] Owing to the exclusion of participants with major cardiac diseases, these associations might be driven by an endogenous inflammation that was independent of myocardial performance and blood pressure, but was probably due to causes such as age, infectious diseases or chronic inflammatory diseases.

The observed unadjusted cross-sectional association between cardiac mass and sTNF-R1 in men, which was weaker in women, is partly consistent with Takei *et al*.[9] However, the inclusion of possible confounders led to a severe decrease in effect estimates in our study, which might be due to the different ethnic and social background of our collective and, thus, a different impact of confounders. Our unadjusted results also agree with the findings of Roselló-Lletí *et al*[10] who described the sTNF-R1 as the most distinctive factor associated with LVH among various blood values related to inflammation (TNF, IL-6, interleukin-1ra, sTNF-R1) in a cohort of asymptomatic hypertensive patients, nevertheless estimates in our study were again lower after covariate adjustment and not associated with LVM, but LADS in men. The weak associations found in the longitudinal analysis are explainable by short-term effects of sTNF-R1, which were previously reported in survival analyses.[7] However, as sTNF-R1 is related to a wide range of inflammation parameters, further inflammatory mechanisms and interactions are likely to be substantial in humans.[29] We found no relevant correlation of sTNF-R1 with the functional parameter of EF. It is possible that the heart might sustain its function by adapting to alterations in the myocardial pattern. Additionally, the EF is characterised by high intrarater and inter-rater variability[30] which limits the significance of EF as a parameter for statistical analyses. We failed to confirm additional associations of sTNF-R1 in the longitudinal analyses and, thus, we could not attribute further predictive value to sTNF-R1.

In women, we found a minimal association of hsCRP with structural echocardiographic parameters, which might reflect a general association of systemic inflammation with cardiac hypertrophy.[31] Using ECG, Bo *et al*[32] has found evidence that cardiac hypertrophy is a 'status of inflammation' causing increased hsCRP levels, which is consistent with our findings as we failed to find considerable longitudinal associations. Differences in estimates and, more importantly, statistical accuracy between men and women is in contrast to previous cross-sectional findings where similar effect sizes between male and female hypertension patients were found.[11] It is likely that in our collective further factors such as kidney function and blood pressure (table 1) have a greater impact on ventricular hypertrophy in men than systemic inflammation represented by plasma hsCRP levels.

The associations of echocardiographic parameters representative for LVH with IL-6 were only minimal, and thus, a considerable association of IL-6 with cardiac hypertrophy was not present in our data, which is consistent with the findings in Takei *et al*.[9]

Comparing echocardiographic parameters between baseline and follow-up we found an apparent improvement at follow-up. This finding might be affected by a coincident improvement in cardiovascular health and most importantly blood pressure (see table 1) reflecting a possible intervention effect due to study participation.

Limitations

Apart from our analyses, further unmeasured inflammation parameters might also be associated with cardiac structure and function.[33] The long interval from baseline to follow-up might conceal short-term effects, which were found in mouse models.[34] In addition, the inter-rater and intrarater variability of echocardiography might have contributed to inconsistent results. In this context, differences in measurement methods between baseline and follow-up, for example, interobserver bias due to different examiners at baseline and follow-up, could have biased the results. Nevertheless, to minimise observer bias, a quality assurance process was implemented during data acquisition and reading, including certification of the echocardiographic examination and reader certification. In addition, all echocardiographic examinations were supervised by a senior cardiologist. Concerning inflammation parameters only hsCRP was measured at follow-up, which limits the ability to assess the change in inflammation parameters in more detail. Despite the effort to adjust for potential confounders, there may be residual confounding factors that were not taken into account. From the clinical point of view, pathological findings in echocardiography without symptoms might not be as relevant as actual diseases, but as complex maladies are often difficult to objectify, we focused on these subclinical echocardiographic values. Finally, despite the attempt to treat missing values adequately in the statistical analysis, we cannot fully exclude a weakening or disruption of results because of missing values.

In conclusion we found a minor cross-sectional association of plasma sTNF-R1 (in both sexes) and plasma hsCRP (in women) with echocardiographic parameters in the general elderly population. Additionally, there are subtle indications of a longitudinal association of sTNF-R1, and hsCRP or IL-6 with LADS. Further studies that survey the changes in cardiac parameters in relation to inflammation, mainly sTNF-R1, over different time intervals and give further insights into the role of inflammation in biochemical processes of heart tissue are now required.[35]

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Competing interests None.

Ethics approval The study was approved by the Ethics Committee of the Medical Faculty of the Martin-Luther-University Halle-Wittenberg and by the State Data Privacy Commissioner of Saxony-Anhalt, and conforms to the principles outlined in the Declaration of Helsinki.

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Inflammation and Prolonged QT Time: Results from the Cardiovascular Disease, Living and Ageing in Halle (CARLA) Study

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Abstract

Background: Previous research found an association of CRP with QT time in population based samples. Even more, there is evidence of a substantial involvement of the tumor necrosis factor-alpha system in the pathophysiology of cardiac arrhythmia, while the role of Interleukin 6 remains inconclusive.

Objective: To determine the association between inflammation with an abnormally prolonged QT-time (APQT) in men and women of the elderly general population.

Methods: Data descend from the baseline examination of the prospective, population-based Cardiovascular Disease, Living and Ageing in Halle (CARLA) Study. After exclusion of subjects with atrial fibrillation and missing ECG recording the final study cohort consisted of 919 men and 797 women. Blood parameters of inflammation were the soluble TNF-Receptor 1 (sTNF-R1), the high-sensitive C-reactive protein (hsCRP), and Interleukin 6 (IL-6). In accordance with major cardiologic societies we defined an APQT above a QT time of 460 ms in women and 450 ms in men. Effect sizes and the corresponding 95% confidence intervals (CI) were estimated by performing multiple linear and logistic regression analyses including the analysis of sex differences by interaction terms.

Results: After covariate adjustment we found an odds ratio (OR) of 1.89 (95% CI: 1.13, 3.17) per 1000 pg/mL increase of sTNF-R1 in women, and 0.74 (95% CI: 0.48, 1.15) in men. In the covariate adjusted linear regression sTNF-R1 was again positively associated with QT time in women (5.75 ms per 1000 pg/mL, 95% CI: 1.32, 10.18), but not in men. Taking possible confounders into account IL-6 and hsCRP were not significantly related to APQT in both sexes.

Conclusion: Our findings from cross-sectional analyses give evidence for an involvement of TNF-alpha in the pathology of APQT in women.

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Introduction

A prolonged QT time is one of the most important electrocardiographic abnormalities, and an important cause of sudden cardiac death [1]. Because the QT time is generally longer in females, women are more often affected by arrhythmia due to a prolonged QT interval than men [2]. Over the last decades, research has mainly revealed insights in the genetic pathogenesis of a prolonged QT time [3]. However, the meaning of further pathophysiological mechanisms in the development of this abnormality is still the subject of research. In particular, the role of inflammation parameters and cytokines has rarely been examined. To our knowledge, there are only a few studies examining the association of inflammation and QT time [4,5]. In a population-based sample, Kim et al. found a positive association of increased blood level of C-reactive protein (CRP) and length of the heart rate-corrected QT time (QTc). This is similar to the results in Kazumi et al. [4], who revealed again a significant association of CRP and QTc in a cohort of young healthy men. While the above-mentioned studies focused mainly on CRP, data examining additional inflammation parameters are still missing. Notably, the soluble tumor necrosis factor receptor 1 (sTNF-R1)

might serve as a promising parameter as it was revealed that sTNF-R1 is a strong predictor for cardiovascular survival [6,7]. In experimental studies, the TNF-alpha system – whose activity can be assessed by the plasma level of sTNF-R1 [8]– was found to influence calcium [9,10] and potassium [11,12] channels affecting QT time (shortening for the former and prolongation for the latter) and the susceptibility to arrhythmia [13]. The stability of sTNF-R1 makes it an easily assessable marker of the larger TNF system [14]. Furthermore, there is evidence from previous studies that interleukin 6 (IL-6) plays a major in role in the pathophysiology of cardiac arrhythmia [15,16].

As prolonged QT time remains often undetected in apparently healthy subjects, it is typically a condition most relevant in (healthy) subjects of the general population, rather than patients in a clinical setting. Thus, the goal of the current study was to analyze the association between inflammation parameters, especially sTNF-R1, and prolonged QT time in the general population.

Methods

Study cohort

We used data from the ***CAR****dio-vascular Disease,* ***L****iving and* ***A****geing in Halle* study (CARLA study), which is a prospective population-based cohort study of the elderly general population of the city of Halle in eastern Germany [17,18]. The CARLA cohort comprises 1,779 participants (baseline response 64.1%) aged 45–83 years at baseline (812 women, 967 men). The baseline examination took place between December 2002 and January 2006. A multi-step recruitment strategy aimed to achieve a high response rate. The percentage final response after subtracting exclusions (individuals who were deceased prior to the invitation, had moved away, or were unable to participate due to illness) was 64%. All data used in this cross sectional analysis descend from the baseline examination of the study. The study participants underwent a detailed medical examination and a standardized, computer-assisted interview, which collected information on socio-demographic and socioeconomic variables, behavioral, biomedical, and psychosocial factors, medical history, and the use of medication within the preceding 7 days. Medication was automatically coded according to the Anatomical Therapeutic Chemical Classification System (ATC code). Additionally, an analysis of non-respondents was performed in order to assess non-response bias by obtaining information about prevalent diseases, and selected behavioral and socio-demographic factors. A more comprehensive account of the CARLA study can be found in Greiser et al. [17]. The study was approved by the Ethics Committee of the Medical Faculty of the Martin-Luther-University Halle-Wittenberg and by the State Data Privacy Commissioner of Saxony-Anhalt and conformed to the principles outlined in the Declaration of Helsinki [19]. All participants gave written informed consent.

Subjects with electrocardiographic signs (n = 44) of atrial fibrillation assessed by Minnesota code [20] and a senior cardiologist were excluded.

Laboratory measurements

Blood samples were taken after a supine rest of 30 minutes. The inflammation parameters of sTNF-R1 and IL-6 were analyzed by the Department of Medicine III, University Clinics Halle (Saale). After a 10-min centrifugation (20°C, 1,500 rpm, Acc = 9, Dcc = 3), the plasma was collected and stored at −80°C. The cytokines were determined using commercially available sandwich enzyme-linked immunosorbent assays (ELISAs: IL-6, Opteia, BD Biosciences, Heidelberg, Germany; TNF-R1, Boehringer Mannheim, Mannheim, Germany).

The determination of CRP was undertaken by the Institute of Laboratory Medicine, Clinical Chemistry and Molecular Diagnostics at the Leipzig University Clinics. The laboratory has been accredited according to the accreditation norms ISO 15180 and ISO 17025. Serum levels of high-sensitivity CRP (hsCRP) were measured using a high-sensitivity immunoturbidimetric method (CRP [Latex] HS, Roche, Mannheim, Germany) on a Hitachi autoanalyzer (Roche Diagnostics, Mannheim, Germany).

Electrocardiogram (ECG) recoding

12-lead ECGs after a supine resting period of at least 20 min were recorded for 10 seconds (sec). All ECGs were processed by the Modular ECG Analysis System (MEANS) [21] to obtain the locations and types of the QRS complexes, and to assess the QT time in the 10-sec ECG. After detecting and, if possible, correcting artefacts by using an algorithm, the program takes all 12 leads into account and computes the QT time from a characteristic beat after considering further beats by an averaging process. Established as an objective method to assess peaks and intervals in ECGs previous studies ascertained the sufficient performance of the MEANS algorithm [22,23]. In an independent test sample the algorithm identified all QRS (dominant type) complexes correctly (one false positive). Evaluating the accuracy of waveform recognition the difference in QT interval duration between MEANS and a reference standard was less than 2 ms with low variation [23].

We corrected the QT (QTc) time for heart rhythm by using the Bazett-Formula [24].

Statistical analysis

According to the recommendations of major cardiologic scientific societies [25], we assumed an abnormally prolonged QT time (APQT) when the QTc was longer than 450 ms in men and longer than 460 ms in women [26]. In this article, we use the term 'abnormally prolonged QT time' rather than 'long QT syndrome', as the latter sometimes refers to underlying genetic abnormalities. Since the heart rate corrected QT time is computed using the uncorrected QT time and the recorded heart rate (HR) we included both parameters additionally in our analyses. All analyses were separately performed in men and women. Using logistic regression analyses with the binary outcome of existing APQT odds ratios with 95% confidence intervals (CI) were estimated as unadjusted and covariate adjusted values. Sex differences in the association of inflammation and QTc were assessed by incorporating an interaction term in the regression models. Non-linearity was assessed using restricted cubic splines which indicated that the assumption of linearity was a sufficient approximation of the exposure-outcome relation [27]. We used linear regression models (unadjusted and adjusted) in the analyses of QTc, QT and HR now as continuous variables. Respecting previous findings [5,28], we adjusted our analyses for age, anti-arrhythmic (ATC code: C01B) and anti-phlogistic medication (ATC code: A07), current smoking status, high density lipoprotein (HDL), cholesterol, glucose blood level, alcohol intake, body mass index, thyroid stimulating hormone (TSH), systolic blood pressure, and potentially QT prolonging drugs (see www.qtdrugs.org) after reevaluating possible confounders by using directed acyclic graphs (DAG) [29]. With the assumed DAG model (see Figure S1) it is possible to estimate the total effect of inflammation on QTc. However, as mediation by electrolytes (not assessed in our study) is likely the direct effect cannot be estimated [30–32].

The adequacy of the considered regression models was assumed when the residuals were normally distributed, which was tested via a Q-Q plot and Cook's distance, which was required to be below

Table 1. Baseline characteristics of the CARLA collective – differentiated according to sex and presence of prolonged QT time.

	Men QTc < 450 ms	Men QTc > 450 ms	p†	Women QTc < 460 ms	Women QTc > 460 ms	p†
	[95% CI]	[95% CI]		[95% CI]	[95% CI]	
N*	796 (46,4%)	123 (7,2%)	-	722 (42.1%)	75 (4,4%)	-
cQT [ms]	417.79 [416.52, 419.07]	468.8 [465.85, 471.78]	-	423.11 [421.77, 424.46]	476.48 [473.03, 479.95]	-
sTNF-R1 [pg/mL]	1159.85 [1129.41, 1191.11]	1230.39 [1133.3, 1335.79]	0.1243	1047.56 [1019.81, 1076.05]	1266.6 [1148.62, 1396.69]	<.0001
IL-6 [pg/mL]	1.68 [1.55, 1.81]	2.26 [1.9, 2.69]	0.0045	1.83 [1.69, 1.97]	2.56 [1.95, 3.36]	0.0106
hsCRP [mg/L]	2.02 [1.86, 2.2]	2.61 [2.21, 3.08]	0.0251	1.85 [1.69, 2.02]	2.38 [1.86, 3.04]	0.0757
BMI [kg/m^2]	27.65 [27.38, 27.92]	28.89 [28.13, 29.66]	0.0014	27.94 [27.57, 28.31]	29.73 [28.63, 30.88]	0.0047
Age [years]	62.87 [62.18, 63.58]	68.36 [66.66, 70.09]	<.0001	62.33 [61.63, 63.04]	66.88 [64.75, 69.09]	0.0002
Syst. BP [mmHg]	143.97 [142.65, 145.3]	150.13 [146.32, 154.05]	0.0013	139.79 [138.19, 141.4]	142.87 [138.53, 147.35]	0.2488
Glucose [mmol/l]	5.87 [5.78, 5.97]	6.33 [6.02, 6.67]	0.0008	5.62 [5.53, 5.7]	5.99 [5.66, 6.35]	0.0102
Cholesterol [mg/day]	5.29 [5.22, 5.36]	5.23 [5.05, 5.41]	0.531	5.63 [5.55, 5.71]	5.67 [5.4, 5.95]	0.7379
HDL [mmol/l]	1.23 [1.21, 1.25]	1.2 [1.15, 1.26]	0.3997	1.51 [1.48, 1.54]	1.51 [1.42, 1.61]	0.9586
Triglycerides[mmol/l]	1.77 [1.71, 1.85]	1.89 [1.72, 2.08]	0.2519	1.42 [1.37, 1.48]	1.48 [1.32, 1.66]	0.5051
TSH [mmol/l]	0.75 [0.71, 0.8]	0.82 [0.73, 0.92]	0.2876	0.75 [0.7, 0.81]	0.65 [0.49, 0.85]	0.1848
Alcohol [g/day]	1.48 [1.07, 2.05]	1.06 [0.44, 2.54]	0.4521	0.02 [0.01, 0.03]	0.04 [0.01, 0.12]	0.3864
	Frequencies					
Antiarrhythmic medication**	5 (0,6%)	1 (0,5%)	0.2187	4 (0,5%)	1 (1,3%)	0.3750
Antiphlogistic medication**	4 (0,5%)	1 (0,5)	0.3750	7 (1%)	1 (1,3%)	0.0703
QT prolonging medication **	89 (11.2%)	25 (20.3%)	0.0042	82 (11.4%)	10 (13.3)	0.6103

Geometric means with respective 95% confidence intervals.
Abbreviation: cQT: Bazett corrected QT interval; sTNF-R1: Soluble tumor necrosis factor type 1; hsCRP: High-sensitive C-reactive protein; IL-6: Interleukin 6; BMI: Body mass index; BP = Blood pressure; HDL: High density lipoprotein; TSH: Thyroid stimulating hormone.
* Proportion referred to the whole sample, ** Proportion within subgroup; †p-values refer to subgroups differences of subject with and without prolonged QT time within sexes.
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one. In order to avoid possible confounding due to hormonal influences in females [33], we performed a sensitivity analysis excluding all premenopausal female subjects (n = 58, self-reported) and women with self-reported intake of sexual hormones (estrogens or gestagens) on a regular basis (n = 28) (detailed results are displayed in the supporting information). Additionally we performed a sensitivity analysis where all subject (120 men and 93 women) with regular intake of potentially QT prolonging drugs (see www.qtdrugs.org) were excluded from the analysis (detailed results are displayed in the supporting information).

The limit of statistical significance was assumed at an α of 5%. All statistical analyses and data management were performed using SAS, Version 9.3 (SAS Inc., Cary, NC, USA).

Missing values

The following parameters incorporated in our analysis contained missing values (the number of missing values is given in brackets): IL-6 (121), hsCRP (82), sTNF-R1 (123), QTc (19), thyroid-stimulating hormone (21), glucose (12), cholesterol (12), HDL (12), triglycerides (12), current alcohol intake (3), and current smoking status (1). All other parameters were measured in all subjects. Using Student's t-Test and Chi-Square Test, we found no statistically significant differences when the mean values or frequencies, respectively, of the considered covariates (age, anti-arrhythmic [ATC code: C01B] and anti-phlogistic medication [ATC code: A07], current smoking status, HDL, cholesterol, glucose blood level, alcohol intake, and atrial fibrillation) were compared between subjects with missing inflammation parameters or QTc and subjects with complete data. As there were few missing values (maximum: 6.97% missing, sTNF-R1) and no evidence of bias or confounding due to incomplete data was evident, we conducted a complete case analysis.

Results

Baseline characteristics

Out of the 1,716 subjects with measured QT time, 123 (13.3%) men and 75 (9.4%) women showed a prolonged QTc according to our definition. Focusing on the inflammation parameters, plasma levels were homogenous between male and female subjects; only in the case of sTNF-R1 men showed higher plasma levels than women (see Table 1). Male subjects with APQT had only slightly higher blood values of sTNF-R1 than men without APQT. However, in female subjects with APQT, we found considerably higher sTNF-R1 plasma levels compared with women without such an electrocardiographic characteristic. The mean values of the inflammation parameter of IL-6 were lower in subjects without APQT then in subjects with APQT. In female and male subjects, hsCRP was higher in the APQT group than in the group with normal QTc (see Table 1). Out of the parameters taken as covariates into account, BMI appeared to be higher in the APQT subgroups of both sexes.

In men, 25 (20.3%) of the subjects with a APQT received potentially QT prolonging drugs (Table 1), while the proportion was distinctly lower in women (13.3%).

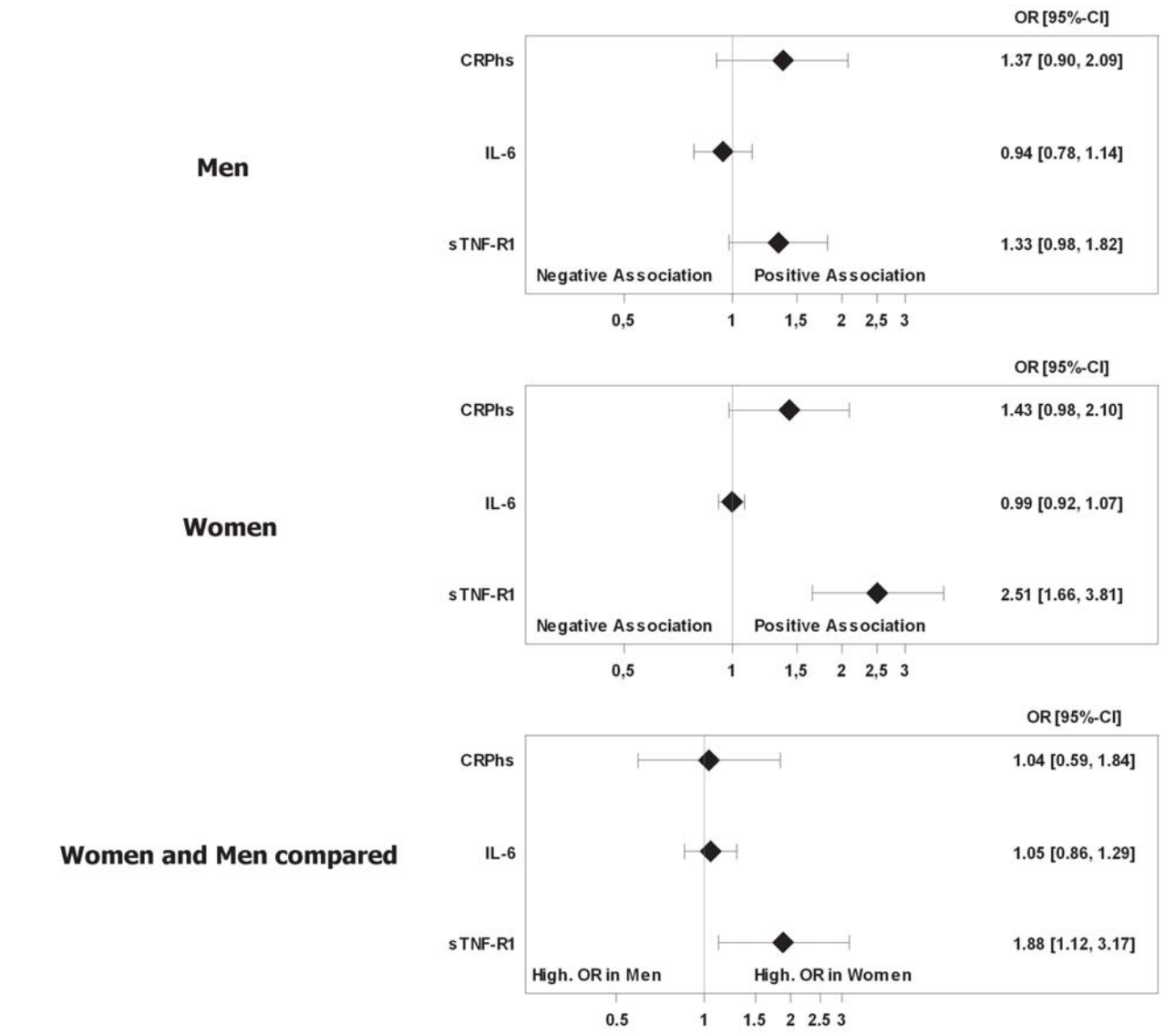

Figure 1. Association of prolonged QTc time and inflammation parameters. Odds ratios (OR) with 95% confidence interval. Unadjusted regression models of inflammation parameter. OR refers to a 1,000 pg/mL increase in sTNF-R1, a 10 pg/mL increase in IL-6, and a 10 mg/L increase in hsCRP.
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Cross-sectional association analysis

Using logistic regression models, sTNF-R1, but not hsCRP and IL-6, had a considerable association with APQT in female subjects. After adjustment for the considered covariates the odds ratio (OR) in women was 1.89 (95% CI: 1.13–3.17) per 1,000 pg/mL increase in sTNF-R1. In contrast, male subjects showed a much lower estimate (0.74, 95% CI: 0.48–1.15), which was accompanied by a considerable amount of uncertainty and was not statistically significant. The interaction analysis supported our finding of a circa 2.5-fold higher OR in women compared with men (OR: 2.55, 95% CI: 1.30–5.02), suggesting a significantly greater chance of APQT with increasing sTNF-R1 in women than in men.

The estimated ORs of hsCRP and IL-6 appeared to reflect null effects, which is contrary to sTNF-R1 in female subjects (Figures 1, 2). Additionally, the results indicated no relevant sex-dependent association of hsCRP or IL-6 and APQT (Figures 1, 2).

Analyzing the QTc as a continuous metric variable in a linear regression model adjusted for possible confounders we found again an association of sTNF-R1 and QTc in women (5.75 ms/

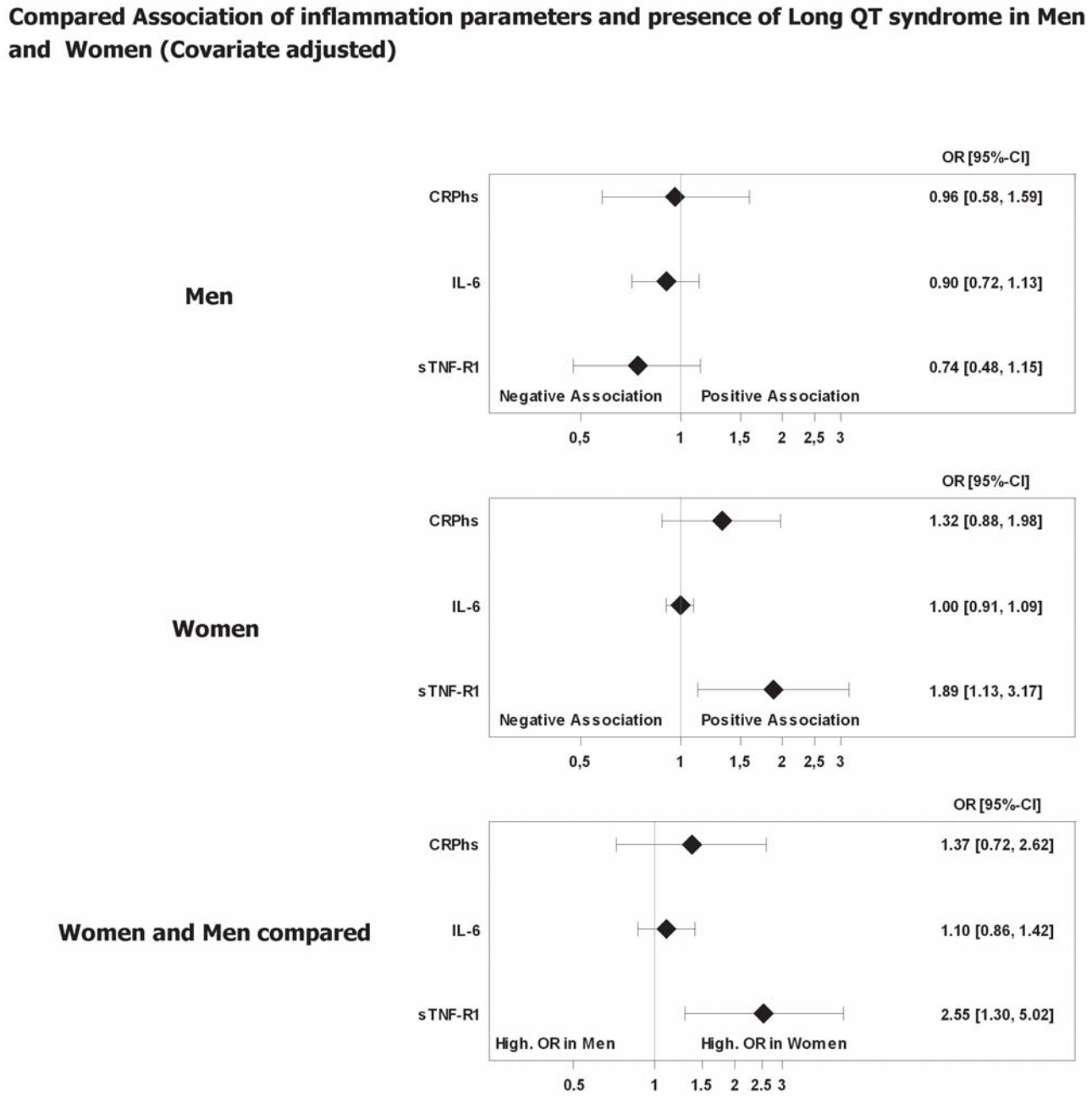

Figure 2. Association of prolonged QTc time and inflammation parameters. Odds ratios (OR) with 95% confidence interval. Models were adjusted for age, anti-arrhythmic (ATC code: C01B) and anti-phlogistic medication (ATC code: A07), current smoking status, high density lipoprotein (HDL), cholesterol, glucose blood level, alcohol intake, body mass index, thyroid stimulating hormone (TSH), systolic blood pressure and potentially QT prolonging drugs (see www.qtdrugs.org). OR refers to a 1,000 pg/mL increase in sTNF-R1, a 10 pg/mL increase in IL-6, and a 10 mg/L increase in hsCRP.

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1000 pg/mL, 95% CI: 1.32–10.18) but not in men (Table 2, Figure 3). The estimated association of QTc and hsCRP in the multiple analysis of both sexes was only minimal with wide confidence intervals (2.17 ms/10 mg/L, 95% CI: —1.96–6.3 in men; 2.02 ms/10 mg/L, 95% CI: −0.23–4.27 in women). In contrast to sTNF-R1, the inflammation parameter of IL-6 was negatively associated with QTc in women independently from possible confounders (0.51 ms/10 pg/mL, 95% CI: 0.09–0.93). In the separate analysis of HR and uncorrected QT time we found a positive association of hsCRP with HR (3.24 s^{-1}/10 mg/L, 95% CI: 1.28–5.2), and consequently a negative relation with the uncorrected QT time in men. Interestingly, neither sTNF-R1 nor IL-6 was associated with either HR or uncorrected QT time in both sexes.

Sensitivity analysis

The exclusion of premenopausal women and women taking oral hormones led to minor changes of the estimates, which were most

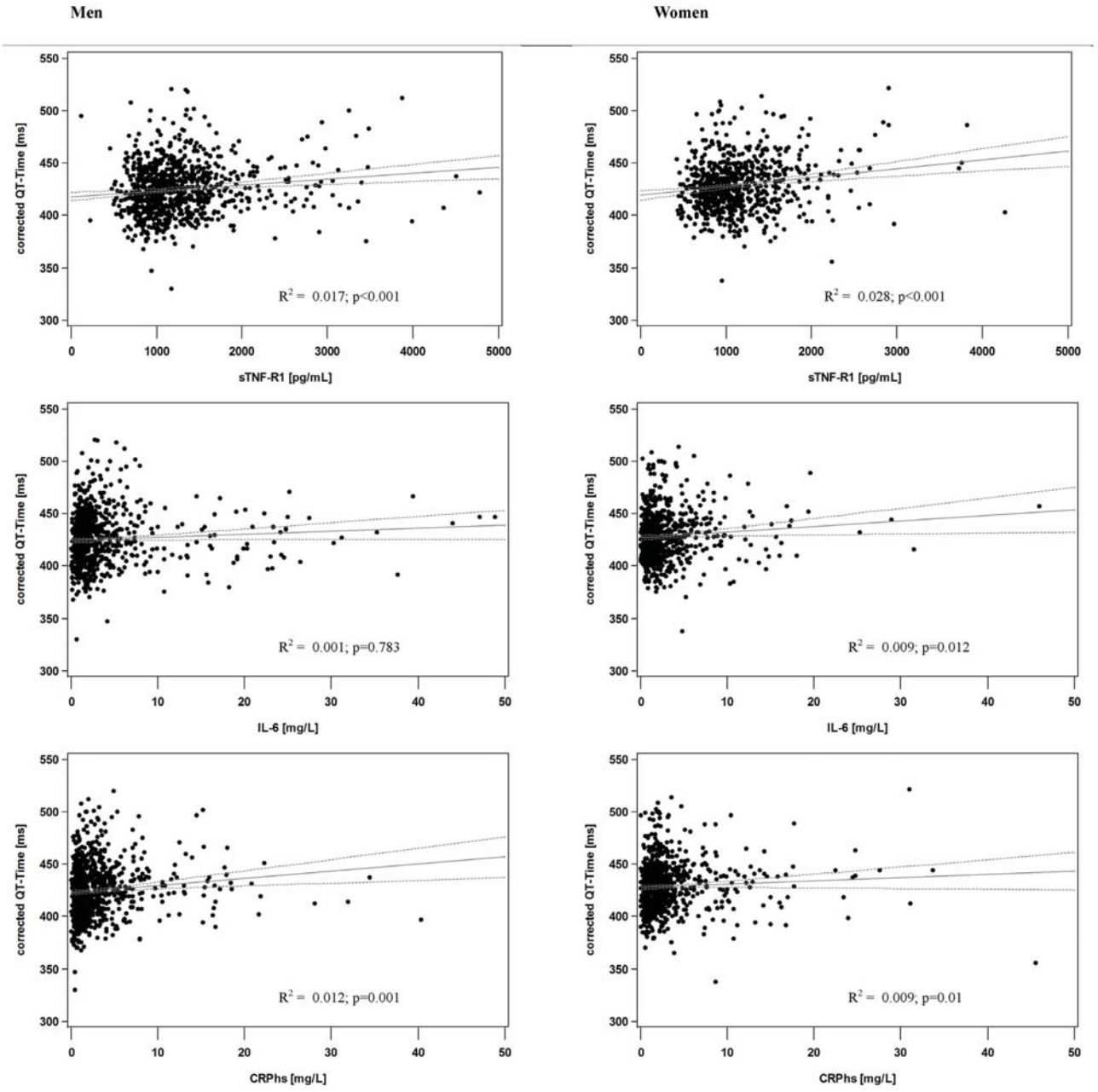

Figure 3. Scatter plot of corrected QT time dependent on sTNF-R1. x-axis: Soluble tumor necrosis factor type 1 (sTNF-R1) in pg/mL; y-axis: heart rate corrected QT time (QTc) in ms.
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relevant in the case of hsCRP where the covariate adjusted association with QTc increased and became statistically significant (see Table S1 and S2). Even more the negative association of IL-6 and QTc after covariate adjustment vanished, while the estimate in the covariate adjusted analysis of sTNF-R1 and QTc increased slightly.

After we excluded subjects with regular intake of potentially QT prolonging drugs effect estimates were not relevantly altered, while they increased slightly when the association of sTNF-R1 with QTc in women was considered (see Table S3 and S4).

Discussion

Summarizing our results, sTNF-R1 seems to be more closely associated with APQT in women than in men, while there was no apparent association of hsCRP or IL-6 with QTc in both sexes. To our knowledge we are the first study that has shown the association of sTNF-R1 and prolonged QT time in women. Additionally, hsCRP was associated with heart rate and thus the uncorrected QT time rather than the actual heart rate corrected QT time. Certain parameters (such as BMI, age) were higher in subjects with APQT underlining their potential role as confounders in the case of a simultaneous effect on exposure and outcome. Considering

Table 2. Linear regression of QTc, QT time and heart rate (HR) on sTNF-R1 in men and women (estimates with 95% confidence interval).

QTc [ms]	Men* [95% CI]	Women*[95% CI]	Men**[95% CI]	Women**[95% CI]
sTNF-R1 [1000 pg/mL]	5.41 [2.52, 8.30]	8.46 [4.81, 12.11]	−0.22 [−3.44, 3]	5.75 [1.32, 10.18]
hsCRP [10 mg/L]	6.8 [2.68, 10.92]	2.94 [0.71, 5.18]	2.17 [−1.96, 6.3]	2.02 [−0.23, 4.27]
IL-6 [10 pg/mL]	−0.11 [−0.93, 0.7]	−0.54 [−0.96, −0.12]	−0.2 [−0.98, 0.59]	−0.51 [−0.93, −0.09]
QT [ms]	**Men* [95% CI]**	**Women*[95% CI]**	**Men**[95% CI]**	**Women**[95% CI]**
sTNF-R1 [1000 pg/mL]	6.13 [2.31, 9.95]	5.71 [0.88, 10.53]	1.54 [−2.73, 5.82]	2.97 [−2.86, 8.79]
hsCRP [10 mg/L]	−5.98 [−11.42, −0.54]	1.42 [−1.45, 4.28]	−8.36 [−13.85, −2.87]	1.14 [−1.77, 4.05]
IL-6 [10 pg/mL]	−1.04 [−2.11, 0.02]	−0.01 [−0.56, 0.54]	−0.95 [−1.99, 0.09]	0.01 [−0.54, 0.56]
Heart Rate [s^{-1}]	**Men* [95% CI]**	**Women*[95% CI]**	**Men**[95% CI]**	**Women**[95% CI]**
sTNF-R1 [1000 pg/mL]	−1.36 [−2.75, 0.02]	0.21 [−1.41, 1.83]	−1.36 [−2.75, 0.02]	0.21 [−1.41, 1.83]
hsCRP [10 mg/L]	3.24 [1.28, 5.2]	0.43 [−0.51, 1.37]	3.24 [1.28, 5.2]	0.43 [−0.51, 1.37]
IL-6 [10 pg/mL]	0.39 [0.02, 0.76]	−0.11 [−0.29, 0.07]	0.39 [0.02, 0.76]	−0.11 [−0.29, 0.07]

Estimates refer to a 1,000 pg/mL increase in sTNF-R1, a 10 pg/mL increase in IL-6, and a 10 mg/L increase in hsCRP.
*unadjusted values; ** covariate adjusted values: models were adjusted age, anti-arrhythmic (ATC code: C01B) and anti-phlogistic medication (ATC code: A07), current smoking status, high density lipoprotein (HDL), cholesterol, glucose blood level, alcohol intake, body mass index, thyroid stimulating hormone (TSH), systolic blood pressure and potentially QT prolonging drugs (see www.qtdrugs.org).
OR refers to a 1,000 pg/mL increase in sTNF-R1, a 10 pg/mL increase in IL-6, and a 10 mg/L increase in hsCRP.
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them as covariates enables us to present results independently from a possibly confounding effect of these parameters (see also directed acyclic graphs in Figure S1). The decrease of effect sizes after covariate adjustment in our analyses might be in part explainable by the presence of confounding in univariate regression models.

Furthermore, we observed a high number of prevalent cases with APQT (11,6% of the entire cohort), which might be mainly due to an equally high prevalence of hypertension and thus ventricular hypertrophy in the CARLA cohort as it was shown previously [34]. Using a QTc cut off of 480 ms Johnson et al. found a prevalence of 13% in their collective of patients with hypertrophic cardiomyopathy [35].

Previous studies reported a considerable relationship between sTNF-R1 and cardiovascular diseases and, more specifically, survival in patients with symptoms of chronic heart failure [6]. It is well established that the TNF-alpha system is involved in the pathophysiology and progression of heart failure [36,37]. Because the shedding of sTNF-R1 from the membrane-bound domain increases as the TNF-alpha system becomes more activated, sTNF-R1 might reflect the activity of TNF-alpha [8,38]. A prolongation of the QT interval has its electrophysiological foundation in a prolonged action potential (AP) of myocardial cells; thus we would expect an increase in AP due to TNF-alpha. There is evidence from animal studies that TNF-alpha causes a decreased expression of various potassium (K^+) channels [11] and a reduction in the density of cardiac transient outward K^+ currents (I_{to}) leading to an increase in AP duration. This mechanism was mediated by the inducible nitric oxide synthase (iNOS) [12], which was itself shown to be influenced by sex hormones (increased after ovariectomy [39]). Furthermore, ion channels (including potassium outward channels) of the human hearts have a different gene-distribution in men and women, which might also include a different susceptibility to TNF-alpha [40]. L-type Calcium channels are a further potential reason for the observed TNF-alpha relations. However, here the results are less conclusive: Recently, it was found that TNF-alpha was associated with a decrease in L-type Calcium currents, which would (contrary to our findings) result in a shortened QT interval [9,10]. In contrast, Duncan et al. [13] reported a Calcium leakage caused by TNF-alpha provoking pro-arrhythmic events. A QT prolonging effect might be triggered, but was not examined by the authors. Summarizing previous experimental data K^+ might play a key role in the observed relations; however these data refer mostly to animal studies rather than human beings. In line with our findings sex differences in the association of sTNF-R1 with cardiovascular events have been reported previously [41]. Interestingly, it was shown in experimental studies that testosterone leads to a decrease in TNF-alpha secretion in human macrophages. As we examined a collective of elderly subects it is unlikely that female hormones serve as an adverse contributor to the found relations in female subjects. Reflecting experimental data the withdrawal of estradiol was accompanied by an increase of TNF-alpha and iNOS [12] expression in ovariectomized rats [42]. This might imply that sex hormones in men and women have an inhibitory effect on the association of TNF-alpha and QT time. In line with this, the exclusion of premenopausal women or women with regular hormone intake caused a minor increase in estimates. However, this effect requires re-evaluation in a separate collective of younger (premenopausal) females to estimate the effect of sex hormones more reliable. In our cohort of the elderly population a direct influence of sex hormones on the estimated effects is less likely. Nevertheless, due to low case numbers in this subgroup we were not able to provide convincing evidence of hormonal effects.

The finding of a negative association of IL-6 with QTc is surprising as a positive association would be expected from previous studies [43]. However, the effect was of low magnitude and the statistical significance might be biased due to the low variance of this parameter in women. Significantly higher levels of CRP in AQPT subjects seem to be driven by the low variance of this parameter rather than a relevant association with QT time, which is underlined by the inconclusive results of regression analyses. A similar explanation might also be true for CRP underpinning that differences below 1 mg/L (and 1 pg/mL in the case of IL-6) are also not relevant from a practical point of view.

Apart from the correlation with heart rate, CRP seems to play a less important role in cardiac electrophysiology than the above-

mentioned sTNF-R1. In one of the few studies having examined the association of inflammation and QT time effect sizes were small [5], which emphasizes the vague role of hsCRP in cardiac arrhythmia [44].

Limitations

As we only used cross-sectional data, we failed to clearly determine the direction of the observed association, that is, whether inflammation caused a prolonged QT time or a long QT time led to higher blood levels of inflammation. At least for hsCRP, Kim et al. [5] have argued that an effect of prolonged QT time on inflammation is imaginable [45]. However, there is humble evidence that inflammation, besides TNF-alpha, influences cardiac ion channels [13], atrial fibrillation [15] and heart rate [46]. Future prospective studies with at least one follow-up might provide deeper insights in the causality of inflammation and prolonged QT time. The observational character of our study makes it difficult to provide deeper insights into underlying (patho)-physiologic mechanisms of our findings.

Furthermore, we only took three inflammation parameters into account. As sTNF-R1 might be implicated in other inflammatory mechanisms and related to a variety of other cytokines [47] further studies are needed that examine a larger variety of possible inflammation parameters. Due to the lack of genetic analyses we were not able to report the effect of inflammation in subjects with genetic anomalies related to a prolongation of QT time. Respecting the collective of the CARLA study our results apply mostly to the elderly population. Further studies with younger (female) subjects are warranted in order to clarify the influence of estrogens on QT time in more detail. Referring to our assumed DAG model (see Figure S1); we are not able to estimate the direct effect of inflammation on QT time (and vice versa) as electrolytes which possibly mediate the effect of inflammation on QT time were not measured in our study. However, it was our primary goal to estimate the total effect of this relation, which is possible with the used regression models [30–32].

In conclusion, our results underline the key role of the TNF-alpha system represented by its receptor in cardiac control circuits. Consequently, the effect of (anti-arrythmic) drugs on inflammation and TNF receptors might appear to be an interesting future research question [48,49].

Supporting Information

Figure S1 Directed acyclic graphs of parameters potentially influencing the association of inflammation and QT time. Minimal sufficient adjustment to estimate the total effect of inflammation of corrected QT time: age, blood pressure, blood fats/cholesterol, QT- prolonging drugs, smoking habit, and thyroid function. Minimal sufficient adjustment to estimate the direct effect of inflammation of corrected QT time: age, blood pressure, blood fats/cholesterol, QT- prolonging drugs, smoking habit, thyroid function, electrolytes.
(TIF)

Table S1 Linear regression of corrected QT time, QT time, and heart rate on inflammation parameters in women after exclusion of premenopausal women and women with regular hormone intake (estimates with 95% confidence interval). Estimates refer to a 1,000 pg/mL increase in sTNF-R1, a 10 pg/mL increase in IL-6, and a 10 mg/L increase in hsCRP. *unadjusted; ** covariate adjusted estimates: models were adjusted for age, anti-arrhythmic (ATC code: C01B) and anti-phlogistic medication (ATC code: A07), current smoking status, high density lipoprotein (HDL), cholesterol, glucose blood level, alcohol intake, body mass index, thyroid stimulating hormone (TSH), systolic blood pressure and potentially QT prolonging drugs (see www.qtdrugs.org).
(DOCX)

Table S2 Logistic regression of APQT on inflammation parameters in women after exclusion of premenopausal women and women with regular hormone intake (odds ratio with 95% confidence interval). Odds ratios refer to a 1,000 pg/mL increase in sTNF-R1, a 10 pg/mL increase in IL-6, and a 10 mg/L increase in hsCRP. *unadjusted Odds ratios; ** Odds ratios adjusted for age, anti-arrhythmic (ATC code: C01B) and anti-phlogistic medication (ATC code: A07), current smoking status, high density lipoprotein (HDL), cholesterol, glucose blood level, alcohol intake, body mass index, thyroid stimulating hormone (TSH), systolic blood pressure and potentially QT prolonging drugs (see www.qtdrugs.org). Abbreviation: APQT = abnormally prolonged QT time.
(DOCX)

Table S3 Linear regression of corrected QT time, QT time, and heart rate on inflammation parameters in men and women after exclusion of subjects with regular intake of potentially QT prolonging drugs (estimates with 95% confidence interval). Estimates refer to a 1,000 pg/mL increase in sTNF-R1, a 10 pg/mL increase in IL-6, and a 10 mg/L increase in hsCRP. *unadjusted; ** covariate adjusted estimates: models were adjusted for age, anti-arrhythmic (ATC code: C01B) and anti-phlogistic medication (ATC code: A07), current smoking status, high density lipoprotein (HDL), cholesterol, glucose blood level, alcohol intake, body mass index, thyroid stimulating hormone (TSH), and systolic blood pressure.
(DOCX)

Table S4 Logistic regression of APQT on inflammation parameters in men and women after exclusion of subjects with regular intake of potentially QT prolonging drugs (odds ratio with 95% confidence interval). Odds ratios refer to a 1,000 pg/mL increase in sTNF-R1, a 10 pg/mL increase in IL-6, and a 10 mg/L increase in hsCRP. *unadjusted Odds ratios; ** Odds ratios adjusted for age, anti-arrhythmic (ATC code: C01B) and anti-phlogistic medication (ATC code: A07), current smoking status, high density lipoprotein (HDL), cholesterol, glucose blood level, alcohol intake, body mass index, thyroid stimulating hormone (TSH), and systolic blood pressure. Abbreviation: APQT = abnormally prolonged QT time.
(DOCX)

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Author Contributions

Analyzed the data: DM. Contributed reagents/materials/analysis tools: HL. Wrote the paper: DM. The analysis of blood samples mainly the analysis of C-reactive protein blood levels: JT. Played a key role in the planing and conduction of the study, and revised the manuscript critically for important intellectual content: AK. Measured the plasma parameters: JT. Participated in the realisation of the study: DM JAK HL JT AK SN DT KHG KW JH. Gave comments: DT AK KHG JK KW JH. Analysed the ECGs: SN. Performed the statistical analyses: DM.

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Prognostic relevance of the interaction between short-term, metronome-paced heart rate variability, and inflammation: results from the population-based CARLA cohort study

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Aims To determine the interaction between HRV and inflammation and their association with cardiovascular/all-cause mortality in the general population.

Methods and results Subjects of the CARLA study ($n = 1671$; 778 women, 893 men, 45–83 years of age) were observed for an average follow-up period of 8.8 years (226 deaths, 70 cardiovascular deaths). Heart rate variability parameters were calculated from 5-min segments of 20-min resting electrocardiograms. High-sensitivity C-reactive protein (hsCRP), interleukin-6 (IL-6), and soluble tumour necrosis factor-alpha receptor type 1 (sTNF-R1) were measured as inflammation parameters. The HRV parameters determined included the standard deviation of normal-to-normal intervals (SDNN), the root-mean-square of successive normal-interval differences (RMSSD), the low- and high-frequency (HF) power, the ratio of both, and non-linear parameters [Poincaré plot (SD1, SD2, SD1/SD2), short-term detrended fluctuation analysis]. We estimated hazard ratios by using covariate-adjusted Cox regression for cardiovascular and all-cause mortality incorporating an interaction term of HRV/inflammation parameters. Relative excess risk due to interactions (RERIs) were computed. We found an interaction effect of sTNF-R1 with SDNN (RERI: 0.5; 99% confidence interval (CI): 0.1–1.0), and a weaker effect with RMSSD (RERI: 0.4; 99% CI: 0.0–0.9) and HF (RERI: 0.4; 99% CI: 0.0–0.9) with respect to cardiovascular mortality on an additive scale after covariate adjustment. Neither IL-6 nor hsCRP showed a significant interaction with the HRV parameters.

Conclusion A change in TNF-α levels or the autonomic nervous system influences the mortality risk through both entities simultaneously. Thus, TNF-α and HRV need to be considered when predicating mortality.

Keywords Heart rate variability • General population • Cardiovascular mortality • CRP • Tumour necrosis factor-alpha • Interleukin 6

Introduction

From its first introduction, heart rate variability (HRV) has been considered as the most valuable non-invasive parameter to assess autonomic control. This is primarily because of its easy measurability and usefulness to assess the activity of the autonomic nervous system function, which was found to be associated with survival.[1–3]

In this context, it is of central interest to identify factors that influence the autonomic nervous system and, thus, measurable HRV

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What's new?

- Results indicating an interaction of the sTNF-R1 representing the TNF-α system and parameters of heart rate variability in the prediction of cardiovascular mortality.
- Assessment of interactions between inflammation and HRV on an additive scale to achieve a closer representation of physiology using recently introduced statistical approaches.
- Considering non-linear HRV parameters to assess the interaction between HRV and inflammation parameters with respect to mortality prediction.

parameters. There is evidence from previous studies that HRV and inflammation are intertwined in terms of a mutual interaction by involvement of vagal reflex circuits as a key component of autonomic response to systemic inflammation.[4] Therefore, assuming a mutual interference of HRV and inflammation and an effect of both entities on cardiovascular survival,[5] we should witness an increase of mortality risk due to an interaction between HRV and inflammation. Such an interaction was previously reported for C-reactive protein (CRP) by Sajadieh *et al.*[6] using community-based data. In addition to CRP, interleukin 6 (IL-6) and the soluble tumour necrosis factor-alpha receptor type 1 (sTNF-R1), the latter as a parameter of the TNF-α system, have been linked to age-related diseases and symptoms in previous studies.[5]

Thus, the primary goal of our study was to evaluate an interaction between HRV and inflammation in terms of their association with general and cardiovascular mortality, while the secondary objective was to assess correlations between HRV and inflammation and their independent association with mortality.

Methods

Study cohort

We used data from the CARdiovascular Living and Ageing in Halle study (CARLA study), which is a prospective population-based cohort study in the general population of the city of Halle, eastern Germany.[7] The CARLA study is an ongoing cohort study of a representative sample comprising 1779 participants (812 women and 967 men) aged 45–83 years at baseline. The baseline examination took place between December 2002 and January 2006. A multi-step recruitment strategy aimed to achieve a high response rate. The final response proportion after subtracting exclusions (individuals who deceased prior to the invitation, had moved away, or were unable to participate due to illness) was 64%. The study participants underwent a detailed medical examination and a standardized, computer-assisted interview. Medication was coded according to the Anatomical Therapeutic Chemical (ATC) classification system. Additionally, an analysis of non-respondents was performed in order to assess non-response bias. Study design and methods have been described elsewhere.[7] Out of 1671 subjects eligible for this study (see below), the final follow-up (from January to October 2013) was performed in 579 men and 519 women. The study was approved by the Ethics Committee of the Medical Faculty of the Martin-Luther-University Halle-Wittenberg and by the State Data Privacy Commissioner of Saxony-Anhalt and conforms to the principles outlined in the Declaration of Helsinki.[8] All participants gave written informed consent.

Electrocardiogram recordings

After a supine resting period of at least 20 min, 20-min electrocardiograms (ECGs) were recorded during metronome respiration at a rate of 15 per minute (0.25 Hz). All ECGs were processed by the Modular ECG Analysis System[9] to obtain Minnesota codes and the locations and types of the QRS complexes (used to compute the standard time and frequency domain parameters of HRV[10]). Artefacts and ectopic beats were replaced by interpolated normal sinus beats. Prior to spectral density estimation, the tachogram of RR intervals was linearly detrended, tapered, and zero padded. Fast Fourier transformation was employed and spectral HRV measures were calculated from the raw periodograms as described previously.[10] The HRV parameters taken into account are given in *Table 1*. All measures of HRV were derived from 5-min segments of the 20-min ECG with a sliding window moving over the ECG in 1-min steps, providing individual HRV parameters for each of the sixteen 5-min segments. For further analysis, we selected the first 5-min segment of each ECG that fulfilled the following quality criteria: <10% of abnormal beats; stationarity of the tachogram according to reverse arrangement tests on the mean and SD of the RR intervals (stationarity is assumed if the *z*-scores are between −2.5 and 2.5); the absence of atrial fibrillation/flutter (AFIB), artificially paced beats and other severe arrhythmias (see below). Electrocardiograms of subjects with extreme HRV values ($n = 39$) were visually reviewed by a cardiologist to identify ectopic beats, abnormal rhythms, or reasons for exclusion which were undetected by computerized classification. The HRV analysis was then repeated for the corrected tachogram, if applicable. A 20-min ECG was recorded for 1777 of 1779 subjects. Finally, 5-min HRVs of 1671 subjects (93% of all 1779 participants) were regarded adequate for further analyses after exclusion of 106 recorded ECGs due to AFIB ($n = 45$), artificial pacemakers ($n = 18$), >10% replaced beats ($n = 27$), other abnormal rhythms ($n = 4$), or technical problems with the ECG recording or processing ($n = 12$).

Parameter assessments

Congestive heart failure was defined in accordance with the algorithm introduced by the European Society of Cardiology, taking clinical symptoms, the plasma levels of NT-pro brain natriuretic peptide (BNP), and echocardiographic findings into account.[12] Plasma levels were assessed as described below. Blood pressure was assessed by taking three consecutive measurements after a resting period of at least 3 min. Information about diabetes, smoking habits, history of myocardial infarctions, sportive activities, and medication intake was acquired using a

Table 1 HRV parameters used for analyses

Time domain parameters	Frequency domain parameters	Non-linear parameters
SDNN	LF power (0.04–0.15 Hz): absolute values; normalized values given in the supplement	SD1 and SD2 from a Poincaré plot[a]
RMSSD	HF power (0.15–0.4 Hz): absolute values; normalized values given in the supplement	SD1/SD2[a]
	The ratio of LF to HF (LF/HF)	Detrended fluctuation analysis (DFA1)[a]

[a]Computed using the software tool gHRV.[11]

computer-based interview and subsequently reviewed by a physician. Weight and height were measured using the SECA-107 digital scale and SECA-220 height measuring system,[7] respectively.

Cause of death

The cause of death was defined as specified in the official death certificate compiled by the Federal Statistical Office. Initially, the cause of death is recorded by a medical doctor and subsequently reviewed by a certified coder at the State Statistical Office of Saxony-Anhalt (Statistisches Landesamt Sachsen Anhalt). For subjects not lost to follow-up, death was recorded from the beginning of the baseline investigation (2002–2006) until the second follow-up (January–October 2013).

Laboratory measurements

Blood samples were taken after a supine rest of 30 min at the baseline examination on the same day as all further examinations, but after all other procedures in the respective subject had been completed. The inflammation parameters of sTNF-R1 and IL-6 were analysed by the Department of Medicine III, University Clinics Halle (Saale). After a 10-min centrifugation (20°C, 1500 rpm, Acc = 9, Dcc = 3), the plasma was collected and stored at −80°C. The cytokines were determined using commercially available sandwich enzyme-linked immunosorbent assays (IL-6, Opteia, BD Biosciences, Heidelberg, Germany; TNF-R1, Boehringer Mannheim, Mannheim, Germany).

The determinations of CRP, creatinine, and other plasma parameters were undertaken by the Institute of Laboratory Medicine, Clinical Chemistry and Molecular Diagnostics, at Leipzig University Clinics. The laboratory has been accredited according to the accreditation norms ISO 15180 and ISO 17025. Serum levels of high-sensitivity CRP (hsCRP) were measured using a high-sensitivity immunoturbidimetric method (CRP [Latex] HS, Roche, Mannheim, Germany) on a Hitachi autoanalyser (Roche Diagnostics, Mannheim, Germany).

Statistical analysis

To assess the correlation between HRV and inflammation parameters, Spearman correlation coefficients (SCCs) were calculated. The association of HRV parameters with survival was tested by estimating hazard ratios (HRs) from Cox regression models (proportionality tested by including a time × variable interaction in the model indicating no significant violation of proportionality; observational period from the baseline examination to the final follow-up). We adjusted for age, sex, metabolic syndrome in accordance with the ATP III definition (non-fasting glucose >10.0 mmol/L was used to define elevated glucose levels),[13] history of myocardial infarction, sport index,[14] plasma level of NT-pro brain natriuretic peptide, HbA1c, low-density lipoprotein, thyroid-stimulating hormone, systolic and diastolic blood pressure, the estimated glomerular filtration rate (using the MDRD-formula[15]), regular intake of anti-arrhythmic medication (ATC: C01Bxxx), beta-blockers (ATC: C07xxxx), digitalis glycosides (ATC: C01Axxx), glucocorticoids (ATC: H02xxxx), antiphlogistic medication (ATC: A07xxxx), thyroid medication (ATC: H03xxxx), and self-reported malignant neoplasms, thyroid disease, or rheumatic diseases. Values were logarithmically transformed in cases of skewed distributions, and standardized. In addition to estimating the (individual) HRV and inflammation effects, we checked for interactions on a multiplicative scale (results reported in the Supplementary material online, *Appendix*) by incorporating interaction terms and main effects. To assess the magnitude of interaction, we calculated the relative excess risk due to interaction (RERI) for continuous variables[16] using the following formula:

$$\mathrm{RERI} = e^{\widehat{\beta_1}+\widehat{\beta_2}+\widehat{\beta_3}} - e^{\widehat{\beta_1}} - e^{\widehat{\beta_2}} + 1,$$

where $\widehat{\beta_1}$ is the HRV estimate from the Cox regression model, $\widehat{\beta_2}$ illustrates the inflammation estimate, and $\widehat{\beta_3}$ stands for the interaction estimate. Beta coefficients of HRV parameters were multiplied with −1, as a negative association of HRV and mortality was presumed.

In contrast to the interaction on a multiplicative scale, the RERI quantifies interactions on an additive scale (departure form additivity).[16] As emphasized previously, biological interactions are best justifiable by a departure from additivity.[16] Regarding the quantitative interpretation, an RERI of 0.5 represents an increase of the HR due to an additive interaction by 50% (confidence intervals above zero indicating a statistically significant risk increase). Confidence limits of the RERI were computed by means of a bootstrapping procedure with 2000 repetitions.[16]

We report the analyses separately for cardiovascular (ICD-10: I00–I99) and all-cause mortality. Hazard ratios are displayed as an increase per standard deviation of the independent parameter with the corresponding 99% confidence interval. The confidence level of 1% (instead of 5%) was chosen to take multiple testing into account (5% significance indicated by '*' in *Tables 3–5* to make findings comparable with studies using this level).

As medication may affect the measurement of HRV, we performed a sensitivity analysis in which all subjects with a regular intake of anti-arrhythmic medication, beta-blockers, and digitalis glycosides (285 men and 282 women) were excluded. In order to differentiate between low- and high-grade inflammation, we conducted a separate sensitivity analysis in which subjects with an hsCRP plasma level >10 mg/L were excluded. Investigating the interaction effect of each inflammation parameter independently from the other, we conducted a further analysis which incorporated all three considered inflammation parameters into one regression model, allowing additionally for interactions between the parameters. Furthermore, HRV parameters from subjects with and without observable movements during the recording were compared (using a Mann–Whitney *U* test).

All statistical analyses were performed using SAS®, Version 9.3 (SAS Inc., Cary, NC, USA).

Results

Baseline clinical characteristics

Table 2 summarizes the baseline characteristics of our study cohort. Of the 1671 subjects enrolled, 226 died during the mean follow-up time of 8.8 years. Among the deceased, the majority ($n = 154$) was men with a mean age at time of death of 76.5 years [99% confidence interval (CI): 74.6–78.5]; in comparison, deceased female subjects ($n = 72$) were characterized by a mean age of 78.4 years (99% CI: 75.7–81.1). Diseases of the cardiovascular system were the cause of death in 44 men and 26 women. Contrary to subjects with low-grade inflammation, subjects with high-grade inflammation had lower means of HRV parameters (see Supplementary material online, *Table SA1*) and higher NT-pro BNP levels, while other cardiovascular risk factors had comparable means.

Correlations analyses of heart rate variability and inflammation parameters

The estimation of correlation coefficients between HRV and inflammation parameters (absolute value <0.21 in all computed correlations) revealed, at most, medium correlations between sTNF-R1, hsCRP, or IL-6 and HRV in our cohort at baseline (*Table 3*). Notable correlations occurred in the cases of sTNF-R1/standard deviation of

Table 2 Baseline characteristics

	All subjects (n = 1671) Mean [99% CI]	Cardiovascular deaths (n = 70, 4.2%) Mean [99% CI]	Survivors (n = 1445, 86.5%) Mean [99% CI]	P-Value[a]
Demographics				
Age (years)[b]	63.9 [63.2, 64.5]	74.4 [71.9, 76.9]	62.6 [61.9, 63.2]	<0.001
HRV parameters				
SDNN (ms)	27.0 [26.2, 27.9]	23.0 [18.6, 28.4]	27.4 [26.5, 28.3]	0.04
LF (ms^2)	174.3 [162.1, 187.5]	98.5 [61.3, 158.3]	182.7 [169.7, 196.7]	<0.001
HF (ms^2)	133.6 [123.3, 144.8]	110.7 [69.4, 176.5]	135.3 [124.5, 147.1]	NS
Ratio LF/HF	1.3 [1.2, 1.4]	0.9 [0.7, 1.2]	1.4 [1.3, 1.4]	<0.001
RMSSD (ms)	19.1 [18.3, 19.9]	19.2 [14.9, 24.7]	18.9 [18.2, 19.8]	NS
Heart rate (s^{-1})	70.7 [69.9, 71.4]	67.0 [63.7, 70.5]	66.9 [66.2, 67.6]	NS
SD1	18.6 [17.6, 19.6]	26.9 [19.3, 37.4]	17.8 [16.8, 18.9]	<0.001
SD2	38.8 [37.6, 40.2]	38.4 [30.7, 47.9]	38.9 [37.6, 40.3]	NS
SD1/SD2	0.5 [0.5, 0.5]	0.7 [0.6, 0.8]	0.5 [0.4, 0.5]	<0.001
DFA1	0.8 [0.8, 0.9]	0.6 [0.5, 0.7]	0.9 [0.8, 0.9]	<0.001
Inflammation parameters				
sTNF-R1 (pg/mL)	1115.1 [1088.3, 1142.6]	1542.9 [1315.3, 1810.0]	1074.2 [1048.0, 1101.1]	<0.001
hsCRP (mg/L)	2.0 [1.8, 2.1]	2.9 [2.1, 4.0]	1.9 [1.8, 2.0]	<0.001
IL-6 (pg/mL)	2.0 [1.9, 2.1]	3.7 [2.7, 5.1]	1.9 [1.7, 2.0]	<0.001
Miscellaneous				
HbA1c (%)	5.8 [5.8, 5.9]	6.2 [5.9, 6.5]	5.8 [5.8, 5.9]	0.01
Sport index	2.3 [2.2, 2.3]	1.9 [1.7, 2.1]	2.3 [2.3, 2.4]	<0.001
NT-pro BNP (pg/mL)	115.2 [107.7, 123.2]	326.7 [213.1, 500.7]	104.7 [97.9, 111.9]	<0.001
Diastolic BP (mmHg)	142.5 [141.2, 143.8]	142.5 [136.1, 149.3]	142.1 [140.7, 143.5]	NS
Systolic BP (mmHg)	84.3 [83.6, 85.0]	77.8 [74.1, 81.8]	84.8 [84.0, 85.5]	<0.001
WHR	0.9 [0.9, 1.0]	1.0 [0.9, 1.0]	0.9 [0.9, 0.9]	NS
LDL (mmol/L)	3.1 [3.1, 3.2]	2.8 [2.5, 3.2]	3.2 [3.1, 3.2]	0.02
eGFR (mL/min)	88.1 [86.6, 89.6]	83.3 [77.3, 89.7]	88.9 [87.3, 90.5]	0.03
TSH (μU/mL)	0.8 [0.7, 0.8]	0.7 [0.5, 1.0]	0.8 [0.7, 0.8]	NS
Frequencies				
Men	893 (53.4 %)	44 (62.9 %)	739 (51.1 %)	NS
Steroides	29 (1.7 %)	1 (1.4 %)	22 (1.5 %)	NS
Diabetes mellitus	246 (14.7 %)	27 (38.6 %)	179 (12.4 %)	<0.001
Rheumatic diseases	351 (22.0 %)	11 (17.5 %)	302 (21.8 %)	NS
Prevalent malignancy	133 (8.0 %)	9 (13.0 %)	95 (6.6 %)	0.05
Antiphlogistic medication	12 (0.7 %)	2 (2.9 %)	8 (0.6 %)	NS
Thyroid-related medication	255 (15.2 %)	10 (14.3 %)	223 (15.4 %)	NS
Anti-arrhythmic drugs	9 (0.5 %)	0 (0.0 %)	9 (0.6 %)	NS
Metabolic syndrome		46 (65.7 %)	566 (39.2 %)	<0.001

Values displayed as their geometric mean because of skewed distributions (except for age).
SDNN, standard deviation of normal-to-normal intervals; LF, low frequency; HF, high frequency; RMSSD, root-mean-square of successive normal-interval differences; SD1, SD2, Poincaré plot; DFA1, short-term detrended fluctuation analysis; sTNF-R1, soluble tumour necrosis factor-alpha receptor type 1; hsCRP, high-sensitivity C-reactive protein; IL-6, interleukin 6; HbA1c, glycated haemoglobin; Sport index, Sport index in accordance to Baecke *et al.*[14]; NT-pro BNP, pro brain natriuretic peptide; BP, blood pressure; WHR, waist to hip ratio; eGFR, estimated glomerular filtration rate; TSH, thyroid-stimulating hormone; NS, not significant.
[a]P-Value for the comparison of survivors with cardiovascular deaths using a Mann–Whitney U test/χ^2 test.
[b]Arithmetic mean.

normal-to-normal intervals (SDNN) ($r = -0.16$), sTNF-R1/low frequency (LF) ($r = -0.19$), sTNF-R1/detrended fluctuation analysis (DFA1) (−0.19), sTNF-R1/SD1–SD2 ratio ($r = 0.21$), and IL-6/LF ($r = -0.13$). In contrast, correlation analyses revealed distinct relationships within HRV parameters, as illustrated in *Table 3*. Correlations between inflammation parameters did not exceed 0.2 in most cases; here, we observed the strongest correlation between hsCRP and sTNF-R1 ($r = 0.23$).

Table 3 SCCs of considered parameters

	sTNF-R1	hsCRP	IL-6	SDNN	LF	HF	LF/HF	RMSSD	SD1	SD2	SD1/SD2	DFA1	Heart rate
TNF-R1	–												
hsCRP	0.23**	–											
IL-6	0.19**	0.14**	–										
SDNN	−0.16**	−0.09**	−0.09**	–									
LF	−0.19**	−0.11**	−0.13**	0.88**	–								
HF	−0.11**	−0.05	−0.07	0.83**	0.70**	–							
LF/HF	−0.11**	−0.06*	−0.05	−0.05*	0.24**	−0.47**	–						
RMSSD	−0.08**	−0.05	−0.06*	0.82**	0.68**	0.93**	−0.42**	–					
SD1	0.06*	0.03	−0.02	0.58**	0.49**	0.66**	−0.29**	0.73**	–				
SD2	−0.09**	−0.05	−0.09**	0.86**	0.80**	0.63**	0.03	0.68**	0.74**	–			
SD1/SD2	0.21**	0.10**	0.04	0.06*	−0.02	0.31**	−0.46**	0.42**	0.78**	0.20**	–		
DFA1	−0.19**	−0.09**	−0.05*	−0.03	0.15**	−0.30**	0.63**	−0.34**	−0.67**	−0.16**	−0.87**	–	
Heart rate	−0.02	0.11	0.12**	−0.31**	−0.25**	−0.42**	0.25**	−0.43**	−0.34**	0.29**	−0.23**	0.19**	–

White background colour: correlation between inflammation parameters; light grey: correlation between HRV parameters; dark grey: correlation between HRV and inflammation parameters.
Abbreviations as in *Table 2*.
Indication of significance: **$P < 0.01$; *$P < 0.05$.

Survival findings

Considering no interactions, covariate-adjusted Cox regression models showed the strongest association of HRV parameters with cardiovascular mortality for LF (HR: 0.8, 99% CI: 0.6–1.0; *Table 4*), SDNN (HR: 0.8, 99% CI: 0.6–1.1) and the non-linear parameters of SD1/SD2 ratio (HR: 1.3; 99% CI: 1.0–1.8) and DFA1 (HR: 0.8; 99% CI: 0.6–1.1), while HRs were <1 in all instances.

When all deaths were taken into account, heart rate was associated with overall mortality (HR: 1.3; 99% CI: 1.0–1.5). Additionally, the SD1/SD2 ratio showed a significant association with general mortality (HR: 1.2; 99% CI: 1.0–1.4) after covariate adjustment.

Regarding inflammation parameters, IL-6 was significantly associated with cardiovascular (HR: 1.5; 99% CI: 1.1–2.1) and overall mortality (HR: 1.2; 99% CI: 1.0–1.5) when confounders were taken into account (*Table 4*), while hsCRP was a stronger predictor of general mortality than IL-6 (HR: 1.3; 99% CI: 1.1–1.5).

Interaction analyses

Regarding the main study focus of HRV/inflammation interactions on an additive scale, covariate-adjusted RERIs of the HRV/sTNF-R1 interaction (*Table 5*) were notable in the case of linear HRV parameters for SDNN (0.5; 99% CI: 0.1–1.0), high frequency (HF) (0.4; 99% CI: 0.0–0.9) and root-mean-square of successive normal-interval differences (RMSSD) (0.4; 99% CI: 0.0–0.9). Thus, it was estimated that the HR increases additively by 0.5 (99% CI: 0.1–1.0) with an increase of 1 SD in sTNF-R1 blood levels and a decrease of 1 SD in SDNN due to the interaction of both parameters. Non-linear HRV parameters indicated no evidence of an interaction with sTNF-R1 in the prediction of mortality (estimated RERIs ranging between −0.1 and 0.2). The analysis of IL-6 showed a numerically considerable interaction with LF (0.5; 99% CI: −0.2 to 1.4), although accompanied by a wide CI. Apart from the LF/HF ratio (0.4; 99% CI: −0.3 to 1.2) there were no RERIs exceeding 0.2 in the analysis of hsCRP.

Sensitivity analysis

When excluding subjects with regular intake of anti-arrhythmic medication, beta-blockers, and digitalis glycosides, the effect estimates changed only slightly, while statistical significance decreased in some analyses due to lower case numbers (see Supplementary material online, *Tables SA6–9*). Relative excess risk due to interactions decreased only slightly when subjects with plasma levels of hsCRP<10 mg/L were taken into account (see Supplementary material online, *Table SA10*). In models including all three inflammation parameters, single effect estimates did not differ significantly from unadjusted, univariate models (change <3%, results not shown). Relative excess risk due to interactions of normalized and absolute LF and HF values were similar (see Supplementary material online, *Table SA5*). Heart rate variability parameters of subjects with and without movements recorded by the study nurse were not significantly different (results not shown).

Table 4 Association of HRV and inflammation parameters with mortality estimated by using separate Cox regression models for each parameter

	Cardiovascular mortality (unadjusted)	Cardiovascular mortality (adjusted)	Overall mortality (unadjusted)	Overall mortality (adjusted)
HRV parameter/heart rate				
SDNN	0.7 [0.5, 1.0]**	0.8 [0.6, 1.1]*	0.8 [0.7, 1.0]**	0.9 [0.8, 1.1]
LF	0.6 [0.4, 0.8]**	0.8 [0.6, 1.0]**	0.7 [0.6, 0.9]**	0.9 [0.8, 1.1]
HF	0.8 [0.6, 1.2]	0.9 [0.6, 1.2]	0.9 [0.8, 1.1]	1.0 [0.8, 1.2]
Ratio LF/HF	0.7 [0.5, 0.9]**	0.8 [0.6, 1.2]	0.8 [0.7, 0.9]**	0.9 [0.7, 1.1]
RMSSD	1.0 [0.7, 1.4]	0.9 [0.7, 1.2]	1.1 [0.9, 1.3]	1.1 [0.9, 1.2]
SD1	1.5 [1.2, 2.0]**	1.1 [0.8, 1.4]	1.4 [1.2, 1.6]**	1.1 [0.9, 1.3]
SD2	1.0 [0.7, 1.3]	0.9 [0.7, 1.2]	1.0 [0.8, 1.2]	1.0 [0.8, 1.1]
SD1/SD2	2.0 [1.5, 2.7]**	1.3 [1.0, 1.8]*	1.7 [1.4, 2.0]**	1.2 [1.0, 1.4]**
DFA1	0.6 [0.5, 0.8]**	0.8 [0.6, 1.1]*	0.7 [0.6, 0.8]**	0.9 [0.8, 1.0]
Heart rate	0.8 [0.6, 1.2]	1.2 [0.8, 1.8]	1.0 [0.8, 1.2]	1.3 [1.0, 1.5]**
Inflammation parameter				
sTNF-R1	2.3 [1.7, 3.0]**	1.2 [0.8, 1.8]	1.9 [1.6, 2.2]**	1.2 [1.0, 1.5]*
hsCRP	1.5 [1.1, 2.0]**	1.3 [1.0, 1.8]*	1.4 [1.2, 1.6]**	1.3 [1.1, 1.5]**
IL-6	1.5 [1.2, 2.0]**	1.5 [1.1, 2.1]**	1.3 [1.1, 1.5]**	1.2 [1.0, 1.5]**

Hazard ratios with 99% confidence intervals. Values are logarithmized and refer to an increase per standard deviation. Models were adjusted for age, sex, metabolic syndrome in accordance with the Program Adult Treatment Panel III guidelines including diabetes mellitus, high-density lipoprotein, triglycerides, hypertension and waist circumference,[13] history of myocardial infarction, sport index,[14] plasma level of NT-pro brain natriuretic peptide, HbA1c blood level, low-density lipoprotein, thyroid-stimulating hormone, systolic and diastolic blood pressure, the estimated glomerular filtration rate (using the MDRD-formula[15]), regular intake of anti-arrhythmic medication (ATC: C01Bxxx), beta-blockers (ATC: C07xxxx), digitalis glycosides (ATC: C01Axxx), glucocorticoids (ATC: H02xxxx), antiphlogistic medication (ATC: A07xxxx), thyroid medication (ATC: H03xxxx), and self-reported malignant neoplasms, thyroid disease, or rheumatic diseases.
SDNN, standard deviation of normal-to-normal intervals; LF, low frequency; HF, high frequency; Ratio LF/HF, ratio of low- to high-frequency power; RMSSD, root-mean-square of successive normal-interval differences; SD1, SD2, Poincaré plot; DFA1, short-term detrended fluctuation analysis; sTNF-R1, soluble tumour necrosis factor-alpha receptor type 1; hsCRP, high-sensitivity C-reactive protein; IL-6, interleukin 6.
Indication of significance: $^{**}P < 0.01$; $^{*}P < 0.05$ (99% CIs include unity in some cases if $0.01 < P < 0.05$).

Discussion

Survival analyses

In the survival analyses, we observed a weak association of HRV with cardiovascular survival in terms of a higher risk as HRV decreases. Simultaneously, we observed a covariate-independent association of IL-6 and hsCRP (the latter not significant under the presumed significance level) with cardiovascular survival.

Our study demonstrated that LF was the HRV parameter characterized by the strongest associations with mortality, as confirmed in a study by Tsuji *et al.*[2] Although, the physiological meaning of this parameter remains unclear, it was eventually attributed to the sympathetic system. The parasympathetic system was found to be represented by the HF, which had only a slightly weaker association with cardiovascular mortality than the LF in our cohort. Thus, an exclusive effect on cardiovascular survival of either system cannot be concluded from our data.[4] Also, relationships of sTNF-R1, hsCRP, and IL6 to cardiovascular mortality have been disclosed by several previous studies.[5]

Correlation and interaction analyses

Correlations analyses between HRV and inflammation parameters revealed notable associations of sTNF-R1 with SDNN, LF, SD1/SD2 ratio, and DFA1, whereby LF correlated modestly with IL-6 plasma levels.

Using metronome-paced, short-term non-linear, and linear HRV parameters to record the activity of the autonomic nervous system,[17] we found an interaction of SDNN, and weaker HF and RMSSD interactions, with sTNF-R1 in its association with cardiovascular survival. Additionally, LF and IL-6 showed a numerically strong interaction, however, accompanied with a considerable statistical uncertainty. Although we found interrelationships between inflammation parameters, the described interactions of sTNF-R1 with HRV parameters appeared to be independent from IL-6 and hsCRP serum levels. The differences in cardiovascular risk factors between subjects with high- and low-grade inflammation could not explain the observed effects.

There is strong evidence from previous studies that inflammation increases vagal tone, while vagal activity is a strong inhibitor of TNF-α release in terms of an inflammation-regulating circuit.[4] The inverse correlation of inflammation (sTNF-R1 and IL-6) and HRV parameters (SDNN and LF) found with our data is of special interest in the light of this vagal reflex circuit. Because, as HF is a measure of the vagus nerve activity, it seems to be the reduced impulse of the vagal nerve that leads to higher serum levels of sTNF-R1 and IL-6, while the inverse causal relationship is unlikely when the theory of an autonomic feedback mechanism is taken into account, as suggested previously.[4] Nevertheless, due to our cross-sectional approach, we cannot rule out the possibility of an inflammation-induced reduction of HRV, as it was shown for IL-6 (most likely due to hypo-responsiveness of the atria rather than increased

Table 5 RERI on an additive scale as described in Knol et al.[16] of sTNF-R1 and HRV parameters with their 99% CI

Interaction	Cardiovascular mortality (unadjusted)	Cardiovascular mortality (adjusted)	Overall mortality (unadjusted)	Overall mortality (adjusted)
sTNF-R1				
SDNN	0.7 [0.0, 1.5]*	0.5 [0.1, 1.0]**	0.2 [−0.1, 0.6]*	0.2 [0.0, 0.4]*
LF	0.7 [0.0, 1.4]**	0.3 [−0.1, 0.8]*	0.3 [0.0, 0.6]*	0.1 [0.0, 0.4]
HF	0.4 [−0.2, 1.3]	0.4 [0.0, 0.9]*	0.1 [−0.2, 0.5]	0.1 [−0.1, 0.3]
LF/HF	0.3 [−0.4, 1.1]	0.0 [−0.6, 0.5]	0.2 [0.0, 0.6]*	0.1 [−0.2, 0.3]
RMSSD	0.4 [−0.3, 1.4]	0.4 [0.0, 0.9]*	0.0 [−0.2, 0.4]	0.1 [−0.1, 0.3]
SD1	−0.2 [−0.7, 0.5]	0.2 [−0.2, 0.6]	−0.1 [−0.3, 0.2]	0.1 [−0.1, 0.3]
SD2	0.1 [−0.5, 0.9]	0.2 [−0.2, 0.6]	0.1 [−0.2, 0.4]	0.1 [−0.1, 0.3]
SD1/SD2	−0.4 [−1.0, 0.2]	0.0 [−0.3, 0.5]	−0.2 [−0.4, 0.1]	0.1 [−0.1, 0.3]
DFA1	0.4 [−0.5, 1.3]	−0.1 [−0.7, 0.7]	0.1 [−0.2, 0.5]	−0.1 [−0.3, 0.2]
Heart rate	0.1 [−0.4, 0.7]	0.0 [−0.3, 0.4]	0.0 [−0.2, 0.3]	0.0 [−0.2, 0.2]
hsCRP				
SDNN	0.0 [−0.6, 0.7]	0.1 [−0.4, 0.8]	0.0 [−0.2, 0.3]	0.0 [−0.2, 0.3]
LF	0.1 [−0.5, 0.9]	0.2 [−0.3, 0.9]	0.1 [−0.2, 0.3]	0.0 [−0.2, 0.3]
HF	−0.1 [−0.5, 0.4]	0.0 [−0.4, 0.7]	0.0 [−0.2, 0.2]	0.0 [−0.3, 0.2]
LF/HF	0.5 [−0.1, 1.2]*	0.4 [−0.3, 1.2]	0.2 [−0.1, 0.5]	0.1 [−0.2, 0.4]
RMSSD	−0.1 [−0.5, 0.5]	0.0 [−0.4, 0.8]	0.0 [−0.2, 0.2]	0.0 [−0.2, 0.2]
SD1	−0.2 [−0.4, 0.1]	0.0 [−0.3, 0.4]	−0.1 [−0.2, 0.1]	0.0 [−0.2, 0.2]
SD2	−0.2 [−0.6, 0.4]	−0.1 [−0.5, 0.5]	−0.1 [−0.3, 0.2]	0.0 [−0.3, 0.2]
SD1/SD2	−0.2 [−0.4, 0.0]	0.0 [−0.3, 0.4]	−0.1 [−0.2, 0.0]	0.0 [−0.2, 0.2]
DFA1	0.2 [−0.2, 0.6]	0.0 [−0.4, 0.4]	0.0 [−0.2, 0.2]	−0.1 [−0.3, 0.1]
Heart rate	0.2 [−0.2, 0.8]	0.1 [−0.4, 0.6]	0.0 [−0.2, 0.2]	0.0 [−0.2, 0.2]
IL-6				
SDNN	0.6 [0.1, 1.5]**	0.4 [−0.2, 1.1]	0.3 [0.1, 0.7]**	0.2 [−0.1, 0.5]
LF	0.9 [0.3, 1.7]**	0.5 [−0.2, 1.4]	0.5 [0.1, 0.8]**	0.2 [−0.1, 0.5]
HF	0.3 [−0.2, 0.9]	0.2 [−0.3, 0.9]	0.2 [0.0, 0.6]**	0.1 [−0.1, 0.4]
LF/HF	0.4 [0.0, 0.8]**	0.3 [−0.2, 1.0]	0.1 [−0.1, 0.4]	0.0 [−0.2, 0.3]
RMSSD	0.3 [−0.2, 1.1]	0.3 [−0.2, 1.1]	0.2 [0.0, 0.5]	0.1 [−0.1, 0.4]
SD1	−0.1 [−0.4, 0.1]	0.0 [−0.4, 0.5]	0.0 [−0.2, 0.2]	0.1 [−0.2, 0.3]
SD2	0.2 [−0.4, 0.9]	0.0 [−0.5, 0.8]	0.2 [−0.1, 0.6]	0.0 [−0.2, 0.4]
SD1/SD2	−0.2 [−0.5, −0.1]	−0.1 [−0.5, 0.4]	−0.1 [−0.2, 0.0]	0.1 [−0.2, 0.3]
DFA1	0.4 [0.0, 1.0]**	0.1 [−0.4, 0.9]	0.1 [−0.1, 0.4]	0.0 [−0.2, 0.2]
Heart rate	0.2 [−0.2, 0.7]	0.2 [−0.4, 0.8]	0.1 [−0.1, 0.3]	0.0 [−0.1, 0.3]

RERI estimated for continuous variables of HRV and inflammation parameters. Models were adjusted for age, sex, metabolic syndrome in accordance with the Program Adult Treatment Panel III guidelines including diabetes mellitus, high-density lipoprotein, triglycerides, hypertension and waist circumference,[13] history of myocardial infarction, sport index,[14] plasma level of NT-pro brain natriuretic peptide, HbA1c blood level, low-density lipoprotein, thyroid-stimulating hormone, systolic and diastolic blood pressure, the estimated glomerular filtration rate (using the MDRD-formula[15]), regular intake of anti-arrhythmic medication (ATC: C01Bxxx), beta-blockers (ATC: C07xxxx), digitalis glycosides (ATC: C01Axxx), glucocorticoids (ATC: H02xxxx), antiphlogistic medication (ATC: A07xxxx), thyroid medication (ATC: H03xxxx), and self-reported malignant neoplasms, thyroid disease, or rheumatic diseases.

SDNN, standard deviation of normal-to-normal intervals; LF, low frequency; HF, high frequency; Ratio LF/HF, ratio of low- to high-frequency power; RMSSD, root-mean-square of successive normal-interval differences; SD1, SD2, Poincaré plot; DFA1, short-term detrended fluctuation analysis; sTNF-R1, soluble tumour necrosis factor-alpha receptor type 1; IL-6, interleukin 6; hsCRP, high-sensitivity C-reactive protein.

Indication of significance: $^{**}P < 0.01$; $^{*}P < 0.05$ (99% CIs include zero in some cases if $0.01 < P < 0.05$).

sympathetic activity) by experimental findings.[18] Similarly, an inverse relationship between IL-6 and HRV (primarily LF and HF) in healthy subjects, which are best comparable with our collective, has been reported previously.[19] Finally, as our LF and HF data were highly correlated, the parasympathetic system seems to contribute considerably to the determination of LF.

When the interaction of HRV parameters with inflammation in terms of mortality is concerned, the mentioned reflex circuit might again be in action. Here, an impaired vagal function might itself increase mortality risk, but it might additionally reduce the anti-inflammatory capacity, which in turn alters the prognostic meaning of inflammation. Using RERIs to assess interactions on an additive scale, such a bio-physiological argumentation, is well supported by our data bearing in mind that additive relationships are common in biology.[16] Interleukin 6 showed a numerically strong RERI, but exhibited wide confidence intervals, which might be due to a

heterogeneous interaction effect between subjects. It is the task of future research to identify determinants which explain this inter-subject heterogeneity. Studies assessing an HRV/inflammation interaction are still sparse. As mentioned, Sajadieh *et al.*[6] revealed an interaction of CRP with HRV, in contrast to our data. One explanation for these differences might be the exclusion of subjects with prevalent heart disease in their study. In conclusion, CRP might not be as important in whole-community samples as it is in subjects free of cardiac diseases.

Non-linear HRV parameters played a less important role in the interaction with inflammation and were not related to mortality, which might be due to usage of daytime 5-min ECGs, rather than a 24 h measurement, as in the study of Stein *et al.*,[19] who also considered heart rate turbulence.

Limitations

The low number of deceased subjects may have hampered the statistical power to detect small effect sizes. Furthermore, death certificates might incorporate a relevant degree of inaccuracy. Nonetheless, such an uncertainty is only of interest if we observed a systematic bias, which is unlikely taking the independency of HRV recording and declaring the cause of death into account. Although we conducted the ECG recording with extensive quality requirements (e.g. by using the described quality criteria), we cannot fully exclude a certain degree of physiological variability, manifesting as a short-term variation in the HRV within the 20-min ECG recording. However, a minimal recording duration of only 5 min is required in epidemiological studies.[20] Furthermore, HRV parameters obtained from a 5-min recording rather than a 24-h recording are not easily comparable. In this context, as we recorded HRV parameters during daytime, we cannot fully exclude environmental influences, e.g. movements,[21] on the HRV measurement, which might be less evident during night-time recording. Nevertheless, there is no evidence that movements influenced HRV values in our study.

Although we recorded HRV parameters by means of paced breathing to achieve a higher reliability than with spontaneous breathing, this method might itself induce a change in the activity of the autonomic system.[17] An upward bias might be present in the interaction analysis with respect to mortality if the measurement error depends systematically on survival, which appears to be unlikely. Findings regarding the correlation analyses might be biased if subjects with high inflammation levels exhibit a stronger/weaker induction of the sympathetic nervous system due to paced breathing than subjects with lower levels. This might account for a portion of the observed correlations, while a systematic effect on several HRV and inflammation parameters appears to be questionable.

The observed interaction is based on statistical grounds, making further studies (including experimental work) essential in order to confirm these findings. Our results apply to the general population above an age of 45 and thus are only generalizable to collectives with a similar age and cardiovascular risk profile. As seriously ill people are less likely to participate, we might witness a bias towards healthier subjects, which would, however, lead to an underestimation of associations of HRV/inflammation with survival and consequently of the estimated interactions.

Conclusion

In conclusion, HRV and inflammation parameters interact in their association with cardiovascular mortality and, thus, should both be considered when assessing mortality risks.

Supplementary material

Supplementary material is available at *Europace* online.

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Different directions of conduction of fascicular potential and ventricular potential during left posterior fascicular ventricular tachycardia ablation: what is the mechanism?

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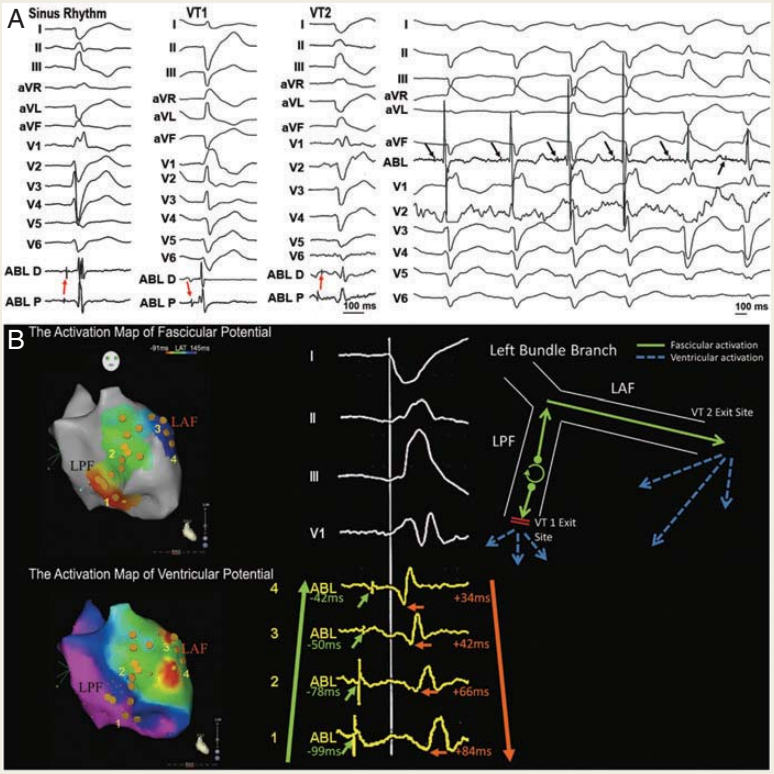

Catheter ablation is a first-line treatment for fascicular ventricular tachycardia (VT). However, insufficient ablation may cause exit site change, which mimic another VT. We illustrated a case of insufficient VT ablation with different exit site. A 49-year-old woman was admitted for electrophysiology study due to palpitation. During the electrophysiology study, the first VT morphology revealed an RBBB pattern favouring a posterior fascicular (LPF) VT. During the LPF ablation, the first VT converted to a second VT, which revealed anterior fascicular-like VT. The activation map of the fascicular potentials and ventricular potentials revealed that the earliest fascicular activation was at the posterior fascicle near the first ablation site, with propagation to the anterior fascicle (LAF), whereas the earliest ventricular activation propagated from the anterior fascicle region to the rest of the ventricle including the substrate near the posterior fascicle. The block distal to the localized-reentry circuit of the first ablation might be created, and the fascicular potential propagated to LAF from LPF to exit. Catheter ablation at the earliest ventricular activation site near the LAF (exit to the ventricle) could not terminate the VT; however, ablation proximal to the earliest ablation site (of the LPF) terminated the tachycardia and the VT became non-inducible thereafter.

The full-length version of this report can be viewed at: http://www.escardio.org/Guidelines-&-Education/E-learning/Clinical-cases/Electrophysiology/EP-Case-Reports.

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QT interval, general mortality and the role of echocardiographic parameters of left ventricular hypertrophy: Results from the prospective, population-based CARLA study

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Abstract

Background: There is convincing evidence of an association between the QT interval on electrocardiograms and general mortality. However, results are inconclusive regarding the extent to which this association depends on ventricular mass and size.

Methods: Data were obtained from the prospective, population-based CARLA study, with a mean follow-up of 8.8 years, after exclusion of subjects with atrial fibrillation (919 men, 797 women aged 45–83 years remained eligible). Echocardiographic parameters were left ventricular mass index, left ventricular diastolic dimension index, diastolic interventricular septum thickness, diastolic left ventricular posterior wall and the relative left ventricular wall thickness. Heart rate-corrected QT interval (QTc) was measured with standard 12-lead electrocardiograms using the MEANS algorithm. The association between QTc and survival was modelled using Cox-regression models (crude- and covariate-adjusted). Values were standardized by dividing the QTc by the standard deviation. The association between QTc and survival was assessed in terms of tertiles of echocardiographic parameters.

Results: In covariate-adjusted models, QTc was associated with general mortality (hazard ratio (HR): 1.19; 95% confidence interval (CI): 1.03, 1.38). Compared with higher tertiles, subjects in the lowest tertile of left ventricular mass index (HR=1.73, 95% CI: 1.26, 2.36) showed the strongest association with general mortality, which was also true for the lowest tertile of diastolic left ventricular posterior wall thickness (HR=1.49, 95% CI: 1.10, 2.02).

Conclusion: In the general population, the association between QTc and general mortality is strongest in subjects with low left ventricular mass index and diastolic thickness of the left ventricular posterior wall, thus the prognostic value of QTc needs to be interpreted with regard to these echocardiographic parameters.

Keywords

Left ventricular mass, QT interval, mortality, general population

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Introduction

In electrocardiography, the heart rate-corrected QT interval (QTc) is of major diagnostic and prognostic interest in clinical and population-based settings. In this context, the association between QTc and total mortality in the general population has been confirmed by several studies.[1] This finding has important implications, as a single, easily measurable parameter might

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add vital information to general risk assessment for mortality. Due to evidence of a positive association between the QT interval and left ventricular hypertrophy (LVH),[2] it is of great relevance to assess the possible interactions of the QT interval with LVH, as the prognostic value of the QT interval may differ with increasing/decreasing left ventricular mass. Two scenarios are possible here: (1) the association between the QT interval and mortality might be higher with increasing ventricular mass due to a mutual effect augmentation; (2) a stronger association between QT interval prolongation and mortality might be present in subjects with low ventricular mass. Assuming that neither a prolonged QT interval nor LVH is preventive, such a situation (stronger association in subjects with low ventricular mass) could arise from LVH blocking the effect of a prolonged QT interval or vice versa, that is, a competing effect on survival in the considered individuals.

Previous research examining the prognostic relevance of the QT interval in relation to ventricular hypertrophy based on clinical collectives[3] or population-based samples[4] used an electrocardiographic assessment (using the Sokolow–Lyon voltage, Cornell voltage, and Cornell product) of LVH, which has the limitation of an indirect measurement. Though changes in the expression of ion channels and a prolongation of the QT interval due to pressure overload-induced LVH have been reported earlier in mice models,[5] to our knowledge, no previous study has assessed the relation between the prognostic value of the QT interval for general mortality and ventricular mass measured using echocardiography in the general population.

Our study aimed to assess the prognostic impact of QT interval prolongation dependent on left ventricular mass in the general elderly population.

Methods

Study cohort

We used data from the CARdiovascular Living and Ageing in Halle study (CARLA study), which is a prospective population-based cohort study in the general elderly population of the city of Halle, eastern Germany.[6] The CARLA study is an ongoing cohort study of a representative sample comprising 1779 participants (812 women, 967 men) aged 45–83 years at baseline. The baseline examination took place between December 2002 and January 2006 (clinical data used for this study were obtained during the baseline examination). A multi-step recruitment strategy was used with the aim of achieving a high response rate. The final response rate after subtracting exclusions (individuals who died prior to the invitation, had moved away, or were unable to participate due to illness) was 64%. The second follow-up was conducted between January 2013 and October 2013 (mean follow-up time = 8.8 years).

The study participants underwent a detailed medical examination and a standardized, computer-assisted interview, which collected information on sociodemographic and socioeconomic variables, behavioural, biomedical and psychosocial factors, medical history, and use of medication within the preceding seven days. Medication was coded according to the Anatomical Therapeutic Chemical Classification System (ATC code). Additionally, an analysis of non-respondents was performed to assess non-response bias by obtaining information about prevalent diseases, selective behaviour and sociodemographic variables. The study design and methods have been described elsewhere.[6] After excluding subjects with atrial fibrillation ($N = 44$) and missing QT interval ($N = 19$) the study cohort comprised 919 men and 797 women. The study was approved by the Ethics Committee of the Medical Faculty of the Martin-Luther-University Halle-Wittenberg and by the State Data Privacy Commissioner of Saxony-Anhalt and conforms to the principles outlined in the Declaration of Helsinki.[7] All participants gave written informed consent.

Electrocardiogram recording

Twelve-lead electrocardiograms (ECGs) were recorded for 10 s after a supine resting period of at least 20 min at baseline. All ECGs were processed by the Modular ECG Analysis System (MEANS). MEANS determines common onsets and offsets for all 12 leads together on one representative averaged beat (identifying the earliest onset of depolarization and the latest end of repolarization across all leads). The duration of the QT interval was measured from the start of the QRS complex to the end of the T wave using a template that was cross-correlated with the ECG signal. MEANS has been extensively evaluated,[8,9] showing the accuracy and stability of its measurements (beat identification of the MEANS algorithm checked for accuracy).

We corrected the QT interval for heart rate using Bazett's formula.[10] To determine LVH electrocardiographically we computed the Sokolow–Lyon (SLI) and the Cornell voltage.

Echocardiographic assessment

At baseline, Doppler echocardiographic examinations were conducted and evaluated by specially trained and certified physicians. All echocardiographers underwent the same dedicated study certification procedures.

For the M-mode examinations, mean intra-observer bias varied between 0.3% and 3.8% (2*SD between 15.3% and 27.7%), while the inter-observer bias ranged between 0.1% and 2.7% (2*SD between 12.7% and 20.8%). All echocardiographic examinations at baseline and follow-up were performed using the GE Vivid ultrasound system (GE Vivid 4 and 5). To quantify the left ventricular (LV) dimensions and function, we chose echocardiographic parameters that are recommended by the guidelines for chamber quantification in echocardiography.[11] The ASE-cube formula was used to calculate the left ventricular mass (LVM), which is in accordance with international guidelines.[11] In addition, the size of the left ventricle was quantified according to the LV diastolic dimension (LVDD) and its index value (LVDDI). The LV-wall thickness was assessed by measuring the diastolic interventricular septum thickness (IVSD) and the diastolic thickness of the posterior wall of the left ventricle (LVPWD). To examine myocardial geometry more closely, we calculated the relative left ventricular wall thickness (LVRWT) according to Lang et al., using the formula $LVRWT = (IVSD + LVPWD)/LVDD$.[11]

Mortality data

Information about dates and causes of death was obtained from the official deaths certificates as registered in mortality statistics. For each death, information such as cause of death encoded by the International Classification of Diseases, Version 10 (ICD-10), place of birth, sex, date of birth and death was available. The cause of death was defined as the underlying disease that began a causal chain leading to death (World Health Organisation definition). All recorded deaths were considered as the study outcome.

Statistical analysis

To give an overview, differences in the mean of baseline variables between subjects with and without signs of prolonged QTc (men $>$ 450 ms, women $>$ 460 ms)[12] were assessed, whereby means are displayed as arithmetic and geometric means, respectively, in the case of skewed distributions (indicated by * in Table 1). Furthermore, differences in general mortality between both groups are displayed in a Kaplan–Meier curve; respective hazard ratios (HRs) were computed using stratified Cox regression (Figures 1 and 2). All subjects were observed from baseline until their second follow-up (censored at the individual time).

Echocardiographic (LVM index (LVMI), IVSD, LVPWD, LVDDI and LVRWT) and electrocardiographic parameters (SLI and Cornell voltage) were categorized into tertiles separately for men and women. Despite the above mentioned classification, QTc was treated as a continuous variable in further analyses, as displayed in Table 2. The association between QTc and overall mortality in each tertile was statistically tested by estimating hazard ratios (confidence limits computed using the Wald approach) from Cox regression models including an interaction term of the respective tertile and QTc (proportionality tested by including a time*variable interaction in the model, which indicated no significant violation of proportionality). Additionally, we tested for non-linear relations by applying restricted cubic splines, which indicated that the linearity assumption was a good fit to the data. Regression models were adjusted for sex, systolic blood pressure, glomerular filtration rate estimated by means of the CKD-EPI formula,[13] HbA1c, total and high-density cholesterol, anti-diabetic drugs/self-reported diabetes mellitus, body surface area heart rate, NT-pro brain natriuretic peptide (NT-proBNP), heart rate, potentially QT prolonging drugs (see www.qtdrugs.org), sportive activity in hours per week, LVMI in non-subgroup analyses, and for interactions of considered covariates and echocardiographic/electrocardiographic tertiles if statistically significant. Covariates were identified using directed acyclic graphs.[14] Several parameters (NT-proBNP, total and high-density cholesterol, heart rate) were logarithmized because of skewed distributions.

Furthermore, to identify a cut-off of a possibly non-linear association of the continuous QTc/LVM interaction with general mortality, we modelled this interaction term by using penalized spline, whereby QTc was assumed to be fixed at one SD above the mean for the cut-off identification after modelling (Figure A1 of the appendix).

As there were no significant differences in QTc between subjects with missing echocardiographic parameters, and no difference in the death rates of subjects with and without missing echocardiographic parameters (results not shown), we conducted a complete case analysis.

We performed the following sensitivity analyses: to compare our data with previous studies, we conducted a sensitivity analysis where the SLI and the Cornell voltage, as ECG measures of LVH, were the exposures (Tables A1 and A2 of the Supplementary Material online). A further sensitivity analysis considered cardiovascular deaths ($N = 72$) as the outcome (Table A3 of the Supplementary Material). Fourth, we performed an analysis where subjects with history of myocardial infarction (Table A4a, A4b, in the Supplementary Material) were excluded ($N = 127$). In addition, we excluded diabetic subjects and subjects with regular intake of potentially QT-prolonging drugs ($N = 423$) using separately the Bazett (Table A5 in the Supplementary Material)

Table 1. Baseline characteristics.

	Unimpaired QTc		Prolonged QTc[a]		
	N	Mean with 95% CI/ *=median (25%–75% quartile)	*N*	Mean with 95% CI/ *=median (25%–75% quartile)	p^b
QTc	1518	420.7 (419.8, 421.6)	198	472.0 (469.6, 474.3)	<0.0001
Echocardiographic Parameters					
LVMI, g/m^2	1435	135.0 (133.1, 136.9)	183	153.8 (147.4, 160.2)	<0.0001
LVRWT, %	1448	47.5 (47.1, 48.0)	185	48.7 (47.5, 50.0)	0.0675
IVSD, cm	1453	1.1 (1.1, 1.2)	186	1.2 (1.2, 1.3)	<0.0001
LVPWD, cm	1452	1.2 (1.1, 1.2)	185	1.2 (1.2, 1.3)	<0.0001
LVDDI, cm/m^2	1456	2.6 (2.6, 2.6)	187	2.6 (2.6, 2.7)	0.0856
Further parameters					
Age, years	1518	63.4 (62.9, 63.9)	198	68.5 (67.2, 69.8)	<0.0001
Sys. RR, mmHg	1517	143.5 (142.4, 144.5)	198	148.9 (145.9, 151.9)	0.0011
Heart rate, beats/min*	1484	65.7 (60.2, 73.2)	187	71.3 (63.0, 81.4)	<0.0001
NT-proBNP, pg/ml*	1468	80.3 (42.4, 152.9)	192	128.7 (66.7, 297.5)	<0.0001
HbA1c, %	1507	5.7 (5.7, 5.8)	197	6.0 (5.8, 6.1)	0.0008
Cholesterol, mmol/l*	1507	5.5 (4.9, 6.2)	197	5.3 (4.8, 6.1)	0.2667
High density cholesterol, mmol/l*	1507	1.4 (1.1, 1.6)	197	1.3 (1.1, 1.6)	0.0443
eGFR, ml/min per 1.73 m^2	1507	86.4 (85.6, 87.2)	197	81.0 (78.4, 83.6)	0.0001
BSA, m^2	1518	1.9 (1.9, 1.9)	198	1.9 (1.9, 1.9)	0.0045
Sportive activity, h	1516	0.8 (0.7, 0.8)	198	0.6 (0.4, 0.8)	0.1767
Number of subjects					
Women	1518	722 (47.6%)	198	78 (39.4%)	0.0123
QT drug	1518	171 (11.3%)	198	35 (17.7%)	0.0140
Diabetes	1517	209 (13.8%)	198	48 (24.2%)	<0.0001

[a]Men > 450 ms, women > 460 ms. [b]*p*-value for differences between unimpaired/prolonged QT time (using Student's *t*-test/Mann–Whitney *U*-test in the case of skewed distributions (*)). *Geometric means. QTc: heart rate-corrected QT interval; CI: confidence interval; LVMI: left ventricular mass index; LVRWT: left ventricular relative wall thickness; IVSD: diastolic interventricular septum thickness; LVPWD: left ventricular diastolic posterior wall thickness; LVDDI: left ventricular diastolic dimension index; RR: blood pressure; NT-proBNP: NT-pro brain natriuretic peptide; eGFR: estimated glomerular filtration rate; BSA: body surface area

and the Fridericia heart rate correction (Table A6 in the Supplementary Material; the latter as it is less susceptible to heart rate) of the QT interval. To assess a possible bias due to a correlation of the Bazett corrected QT interval with the RR interval more closely, we compared the mean of tertiles of echocardiographic/electrocardiographic LVH parameters using an analysis of variance (Figure A2 in the supplement). Furthermore, the squared Pearson correlation coefficient of the relation of the RR interval/Bazett corrected QT interval, the RR interval/QT interval, and the RR interval/Fridericia corrected QT interval (Figure A3 in the Supplementary Material) were computed. We set a *p*-value threshold of 0.05 for statistical significance. All statistical analyses and data management were performed using SAS®, Version 9.3 (SAS Inc., Cary, NC, USA) and R version 3.0.2.[15]

Results

Descriptive results

We observed higher LVMI, IVSD and LVPWD in subjects with prolonged QTc (see above) than in subjects without (Table 1). Subjects with QTc prolongation were older, had a higher systolic blood pressure, heart rate, body surface area (BSA), plasma levels of NT-pro BNP, and HbA1c, while the estimated glomerular filtration rate (eGFR) was reduced in this group. Additionally, this group contained more diabetic subjects and subjects with a regular intake of potentially QT-prolonging drugs.

As shown in Figure 1, a prolonged QTc was associated with a worse overall survival (crude HR=2.96, 95% confidence interval (CI): 2.22–3.96; covariate adjusted HR=1.59; 95% CI: 1.11–2.26). During the observational period, 164 men and 77 women died.

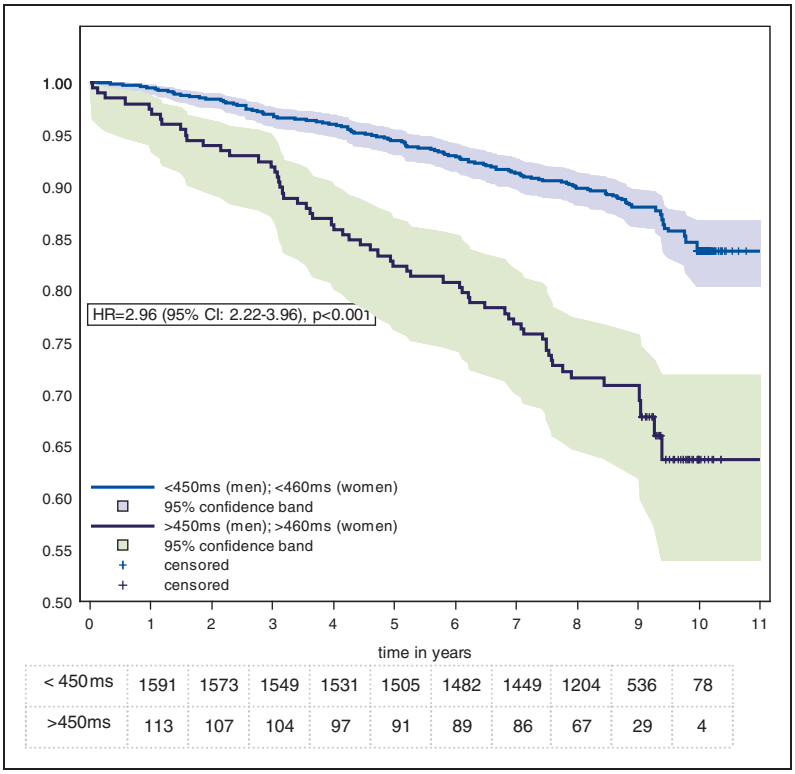

Figure 1. Kaplan–Meier curve of subjects with and without indications of prolonged QT interval (QTc > 450 ms in men and >460 ms in women). HR obtained from Cox regression.
QTc: heart rate-corrected QT interval; HR: hazard ratio; CI: confidence interval

Association of QTc with general mortality in relation to echo- and electrocardiographic parameters of LVM

We found the strongest association between QTc and general mortality after covariate adjustment in the lowest tertile of LVMI compared with both other respective tertiles (Table 2) when covariates were considered (HR=1.73; 95% CI: 1.26, 2.36). This is confirmed by a strongly diverging Kaplan–Meier curve in the lowest tertile of LVMI when compared with both remaining tertiles (Figure 2, see Figure A4 for the Fridericia correction). Similarly, subjects with the lowest values of LVPWD were characterized by a considerably stronger association between QTc and general mortality than subjects with higher LVPWD values. A linear trend of decreasing effect estimates with increasing tertiles of echocardiographic parameters was significant for LVMI and LVPWD.

On a continuous scale, the predicted risk increased exponentially below a LVMI of 145.1 g/m^2 (Figure A1 of the Supplementary Material) when the QTc interval was fixed at 1 SD above the mean.

Using an electrocardiographic determination of LVH we found equally strong effect estimates between the respective tertiles after covariate adjustment.

Sensitivity analyses

The sensitivity analysis assessing the association of the SLI and Cornell voltage with general mortality disclosed no relevant prognostic value of both parameters (Tables A1 and A2 in the Supplementary Material) in our cohort. When deaths due to cardiovascular causes were considered effect estimates were generally larger, while still the lowest tertile, apart from relative posterior wall thickness (RWT), exhibited the strongest association with mortality (Supplementary Material Table A3). Similarly, the exclusion of subjects with history of myocardial infarction, diabetes and regular intake of potentially QT-prolonging drugs did not change effect estimates and relations relevantly (Supplementary Material Tables A4a, A4b and A5). This was also true when the QT interval corrected by means of the Fridericia formula was taken into account, though, compared with Bazett's formula, group differences decreased slightly in the case of LVMI (Supplementary Material Table A6), which is best explained by a flatter slope in the non-linear model above 145g/m^2 (Supplementary Material Figure A1). RR intervals differed between tertiles of LVMI, LVDDI, RWT, SLI and the Cornell voltage, but not IVSD and LVPWD (Supplementary

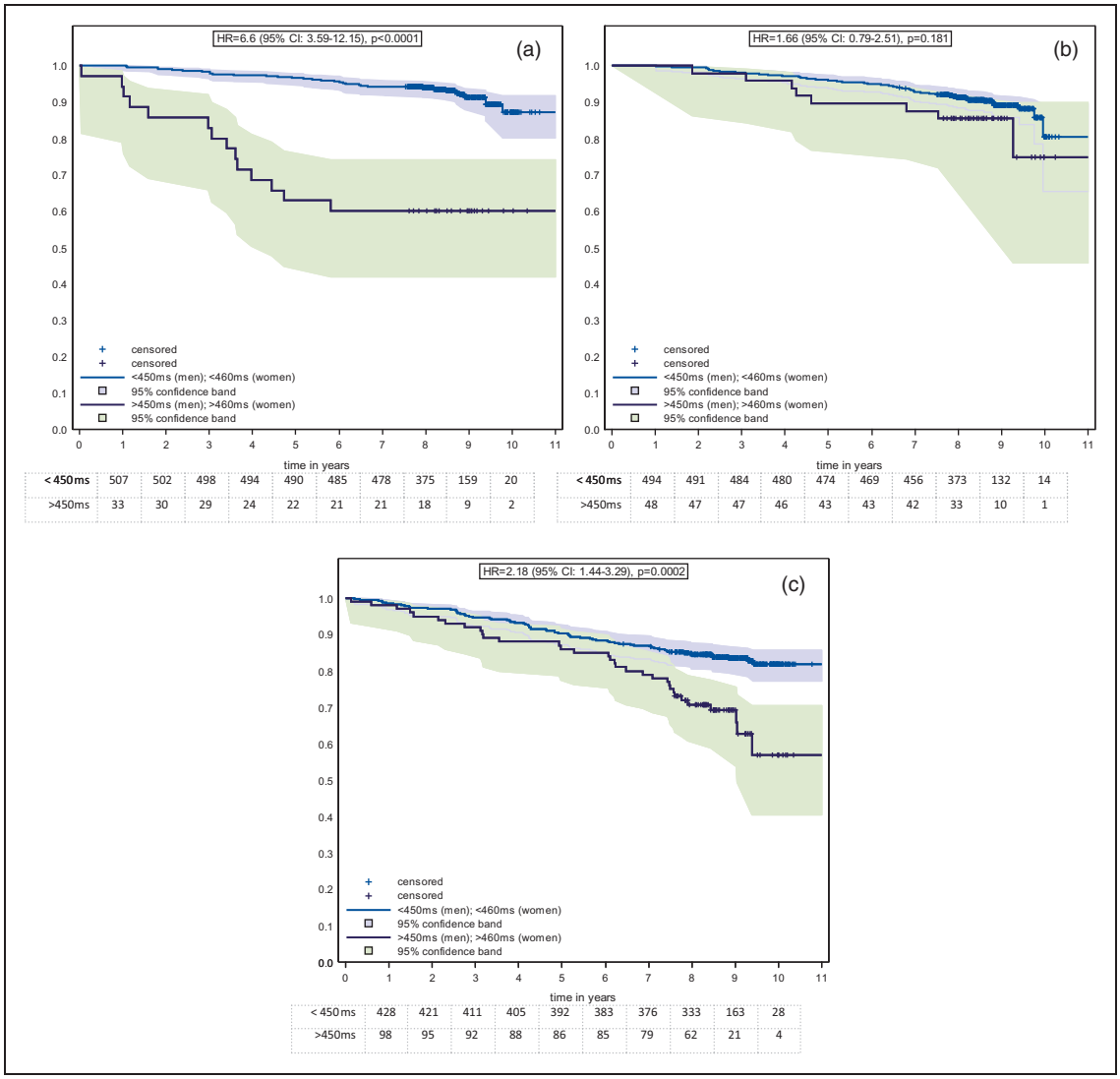

Figure 2. Kaplan–Meier curve of subjects with and without indications of prolonged QT interval (QTc > 450 ms in men and >460 ms in women) in tertiles of left ventricular mass index ((a) lowest tertile, (b) middle tertile, (c) highest tertile). HR obtained from Cox regression.
QTc: heart rate-corrected QT interval; HR: hazard ratio; CI: confidence interval

Material Figure A2) making a heart rate induced bias of observed group differences in the predictive value of the QTc unlikely.

The lowest correlation between the RR interval and (corrected) QT intervals was found when the Fridericia correction formula was applied (Supplementary Material Figure A3), while the Bazett correction disclosed a low correlation with the RR interval ($r^2 = 0.154$).

Discussion

We found an increasing association between QTc and general mortality in subjects with decreasing ventricular mass or wall thickness of the left ventricle. The observed relations were robust in the performed sensitivity analyses. These findings confirm parts of the results by Haugaa et al.,[3] who found a better survival in patients with a QTc between 450 ms and 499 ms and concomitant LVH, but the relationships were inverted in patients with a QTc above 500 ms. This subpopulation (QTc > 500 ms) was only weakly represented in our study population (13 cases, five deaths), and thus contributed little to our results. Furthermore, the collectives differ considerably between the studies (clinical in Haugaa et al.[3] versus general elderly population in our study). Thus, a QTc above 500 ms in cardiac patients might have a very different meaning from the same QTc in the general population (due to drug administration in patients and concomitant diseases).

Table 2. Effect estimates from Cox regression of the association of the QTc interval with survival in respect to echocardiographic parameters.

	Unadj.	*p*-inter. (obs. used)	Adj.	*p*-inter. (obs. used)
All	1.5 (1.33, 1.68)	–	1.19 (1.03, 1.38)	–
LVMI				
Lowest tertile	1.89 (1.45, 2.47)	0.073 ($n = 1633$)	1.73 (1.26, 2.36)	0.024 ($n = 1547$)
Medium tertile	1.30 (0.98, 1.72)		1.09 (0.82, 1.44)	
Highest tertile	1.34 (1.14, 1.56)		1.06 (0.88, 1.28)	
RWT				
Lowest tertile	1.66 (1.28, 2.15)	0.215 ($n = 1633$)	1.32 (0.98, 1.79)	0.360 ($n = 1547$)
Medium tertile	1.51 (1.22, 1.87)		1.12 (0.89, 1.41)	
Highest tertile	1.37 (1.14, 1.65)		1.11 (0.92, 1.35)	
IVSD				
Lowest tertile	1.60 (1.19, 2.15)	0.615 ($n = 1639$)	1.51 (1.08, 2.10)	0.209 ($n = 1552$)
Medium tertile	1.37 (1.09, 1.72)		1.08 (0.84, 1.39)	
Highest tertile	1.43 (1.21, 1.69)		1.12 (0.93, 1.34)	
LVPWD				
Lowest tertile	1.87 (1.42, 2.46)	0.037 ($n = 1637$)	1.49 (1.10, 2.02)	0.028 ($n = 1550$)
Medium tertile	1.46 (1.16, 1.84)		1.25 (0.96, 1.63)	
Highest tertile	1.31 (1.10, 1.55)		1.03 (0.86, 1.24)	
LVDDI				
Lowest tertile	1.65 (1.34, 2.04)	0.241 ($n = 1643$)	1.27 (1.01, 1.61)	0.049 ($n = 1554$)
Medium tertile	1.46 (1.14, 1.85)		1.33 (1.02, 1.73)	
Highest tertile	1.39 (1.16, 1.67)		0.96 (0.79, 1.18)	
Electrocardiographic parameters of LVH				
Sokolow–Lyon index				
Lowest tertile	1.72 (1.43, 2.08)	0.202 ($n = 1716$)	1.11 (0.91, 1.37)	0.874 ($n = 1615$)
Medium tertile	1.24 (0.97, 1.60)		0.93 (0.71, 1.22)	
Highest tertile	1.46 (1.23, 1.73)		1.10 (0.91, 1.33)	
Cornell voltage				
Lowest tertile	1.69 (1.37, 2.09)	0.110 ($n = 1716$)	1.05 (0.84, 1.32)	0.862 ($n = 1615$)
Medium tertile	1.52 (1.20, 1.93)		1.14 (0.87, 1.48)	
Highest tertile	1.36 (1.15, 1.61)		1.09 (0.90, 1.31)	

Association of QTc time with general mortality in tertiles of respective echocardiographic parameters. Hazard ratios with 95% confidence intervals describe the relative hazard change by increase of 1 SD in respective measures. Models adjusted for sex, systolic blood pressure, glomerular filtration rate estimated by means of the CKD-EPI formula,[13] HbA1c, total and high-density cholesterol, anti-diabetic drugs/self-reported diabetes mellitus, body surface area heart rate, NT-pro BNP, heart rate, potentially QT prolonging drugs (see www.qtdrugs.org), sportive activity in hours per week.
QTc: heart rate corrected QT time; NT-proBNP: NT-pro brain natriuretic peptide; Unadj.: unadjusted estimates; Adj.: adjusted estimates; LVMI: left ventricular mass index; RWT: relative posterior wall thickness; IVSD: interventricular wall thickness (diastolic); LVPWD: left ventricular posterior wall thickness (diastolic); LVDDI: left ventricular diastolic dimension; LVH: left ventricular hypertrophy; *p*-inter.: p-value for linear interaction of increasing/decreasing effect estimates as tertiles rise

Third, an echocardiographically diagnosed LVH might be distinct from an assessment of LVH by ECG (using methods such as the SLI).[3,4] This is especially important because the agreement of ECG measures of LVH with echocardiography was found to be weak;[16] and the SLI and the Cornell voltage showed only a weak association with mortality and no interaction with the QTc in our cohort.

In our population, interventricular and posterior wall thickness (IVSD/LVPWD) appeared to be the parameters that mainly contributed to the observed relationships (stronger association between QTc and survival as LVM decreases), while the prognostic value of QTc differed less between LVDDI categories. Similarly, previous research found a stronger association between parameters related to repolarization disturbances and parameters of wall thickness than with left ventricular dimension.[2] Additionally, the same study found an independent effect of LVMI on ventricular repolarization. As mentioned, changes in the

expression of repolarizing K^+ channels due to pressure overload-induced LVH have been found in mice models causing also a prolongation of the QT interval.[5] Considering our findings, such alterations might induce a QT prolongation, which has little predictive value for general mortality in the general population. The changes induced by cardiac hypertrophy (e.g. decrease in the density of slow K^+ channels[5]) might lead to an action potential that is sparsely alterable by non LVH induced pathologies (such as inflammation), which, nevertheless, influence survival. In this context, the QT interval has been related to systemic conditions such as inflammation,[17] which is itself caused by a wide variety of diseases. Thus, the QT interval in subjects without signs of LVH might reflect diseases and conditions with a ubiquitous, adverse effect on the human body. This competitive relation between QT prolongation related to ventricular mass and QT prolongation due to other causes may explain a weaker association between QTc and overall mortality in subjects with higher LVM. In such a scenario, both forms should be considered to be separate entities linked to distinct pathogenesis, with QT prolongation not due to LVH incorporating a stronger prognostic meaning for general mortality.

None of the deaths in our cohort was coded as sudden cardiac arrest, making arrhythmia unlikely to be a major contributor to our findings. As we adjusted for potentially QT prolonging medication, a confounding effect of drug intake is also unlikely. At the cellular level, the accumulation of collagen and the related fibrosis was identified as a possible mechanism resulting in prolongation of the QT interval.[18]

These findings have the following clinical implications: QTc is a suitable marker for overall survival in the general population. However, this prognostic value should be seen in the context of LV mass and wall thicknesses. That is, the strongest prognostic value is seen in subjects with low LV mass and wall thickness.

Limitations

The limitations of our study include its consideration only of an elderly cohort of the general population, which makes it impossible to assess the implication of QTc and ventricular mass on mortality in younger subjects. Furthermore, heritable hypertrophic cardiomyopathy, which is relatively rare in the general population (approximately 1 in 500), should be considered a separate entity and not mixed in with an increase in cardiovascular mass due to ageing and concomitant diseases such as arterial hypertension, which were most relevant in our cohort.[19] As family history of hypertrophic cardiomyopathy or sudden cardiac death at an early age was not recorded in our study, we are unable to assess the predictive value of the QT interval in heritable forms of ventricular hypertrophy. The same applies to a prolongation of QT interval that can be ascribed to genetic alterations of ion channels.[20]

Furthermore, we took the entire QT interval into account without separating it into its parts, that is, a potential prolongation of the QRS complex and/or the JTc interval; however, the former represents depolarization, while the latter is related to repolarization, and considering the intervals separately could reveal different prognostic impacts. As mentioned earlier we cannot estimate the effect of QTc and ventricular mass in subjects with a very long QTc, for example, above 500 ms, as only five out of the 241 deaths showed this feature (four showed signs of congestive heart failure or took QT prolonging drugs). However, these subjects exhibit the greatest risk of arrhythmias and sudden cardiac death.[19]

In conclusion we found that the prognostic relevance of QTc to overall survival is most pronounced in subjects with low (posterior) ventricular wall thickness and, thus, low ventricular mass.

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Conflict of interest

The authors declare that there is no conflict of interest.

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Thesen

(1) Hinsichtlich möglicher Ursachen einer erhöhten Entzündungslast erscheint besonders das viszerale Fettgewebe ein Ort starker chronischer Entzündungsaktivität zu sein.

(2) Gleichzeitig zeigten frühere Arbeiten (Knopf *et al.* 1999), dass Übergewicht eine erhöhte Prävalenz in Bevölkerungsschichten mit geringem Bildungsniveau hat.

(3) Diese hypothetische kausale Kette soll analysiert werden: Personen mit einem geringeren Bildungsniveau zeigen ein höheres Entzündungsniveau.

(4) Eine zu bestimmende Proportion dieses Effekts wird dabei über anthropometrische Parameter vermittelt, die mit Übergewicht in Verbindung stehen. Modellhaft würden also die anthropometrischen Parameter als Mediatoren des Effekts des Bildungsniveaus auf den Grad der Entzündung dienen.

(5) Eine analoge Argumentation ist hinsichtlich herzstruktureller Parameter zu führen. In Analogie zur Fragestellung (1) soll ebenfalls die Assoziation des Bildungsniveaus mit echokardiografischen Parametern der linksventrikulären Masse überprüft werden. Auch hier ist eine Quantifizierung dieses Effektes mit Hilfe einer Mediatoranalyse möglich.

(6) Inflammationsparameter (CRP, IL-6, sTNF-R1) sowie echo- und elektrokardiografische Parameter der linksventrikulären Hypertrophie zeigen eine Assoziation im Längs- und Querschnitt.

(7) In dem untersuchten Kollektiv konnte eine schwache Assoziation der echo- und elektrokardiografischen Parameter nur im Querschnitt gezeigt werden.

(8) Aufgrund ihrer engen Verbindung sollen QT-Zeit und echokardiografische Parameter, hier besonders die linksventrikuläre Masse, eingehender untersucht werden. Unter Berücksichtigung einer möglichen Beziehung zwischen QT-Zeit und Herzmasse ist zu argumentieren, dass der Effekt der QT-Zeit als entscheidender Risikofaktor für den plötzlichen Herztod von dem Grad der linksventrikulären Hypertrophie beeinflusst wird.

(9) Im epidemiologischen Kontext stellt sich bei einer solchen Konstellation die Frage nach einer möglichen Interaktion zwischen beiden Parametern hinsichtlich eines harten Zielkriteriums, wie der Gesamt- bzw. kardiovaskulären Mortalität.

(10) Der prädiktive Wert des sTNF-R1 in Bezug zu Mortalitätsendpunkten ist bisher nicht in einem populationsbezogenen Kontext untersucht worden. Analog zum CRP wäre hier mit einem risikoerhöhenden Effekt des sTNF-R1 bei Betrachtung der kardiovaskulären oder Gesamtmortalität zu argumentieren.

(11) Die Interaktion des autonomen Nervensystems mit Inflammationsparametern soll hinsichtlich des Endpunkts der kardialen und allgemeinen Mortalität untersucht werden.

(12) Zur Quantifizierung der Aktivität des autonomen Nervensystems soll als nicht-invasive Methode die Herzfrequenzvariabilität (HRV) zur Anwendung kommen.

(13) Der lösliche TNF-Rezeptor zeigte eine Assoziation bezüglich der Mortalität in der Allgemeinbevölkerung.

(14) Die Vorhersagekraft des löslichen TNF-Rezeptors hinsichtlich der Mortalität ist abhängig von der Aktivität des autonomen Nervensystems, gemessen mit Hilfe der Herzfrequenzvariabilität.

(15) Die stärkste Interaktion zeigt sich bei Parametern des autonomen Nervensystems.

Eidesstattliche Erklärung

Ich erkläre an Eides statt, dass ich die Arbeit selbstständig und ohne fremde Hilfe verfasst habe. Alle Regeln der guten wissenschaftlichen Praxis wurden eingehalten; es wurden keine anderen als die von mir angegebenen Quellen und Hilfsmittel benutzt und die den benutzten Werken wörtlich oder inhaltlich entnommenen Stellen als solche kenntlich gemacht.

Halle (Saale), den 16. Mai 2018

Daniel Medenwald

Erklärung über frühere Habilitationsversuche

Ich erkläre, dass ich mich an keiner anderen Hochschule einem Habilitationsverfahren unterzogen bzw. eine Habilitation begonnen habe.

Halle (Saale), den 16. Mai 2018

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