

Arterial Function, Apelin and Atherosclerotic Cardiovascular Disease in Patients with Rheumatoid Arthritis

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Declaration

This is to certify that this thesis is my own work and has not been submitted before for any degree or examination in this or any other University. I have worked intensively together with my supervisors Professor Patrick Dessein and Associate Professor Aletta Millen in the process. As part of a team of researchers at Milpark Hospital and Charlotte Maxeke Johannesburg Academic Hospital, including Prof Aletta Millen, Ms Chanel Robinson and Ms Lebogang Mokotedi, we performed all applanation tonometry and echocardiography assessments. Prof Patrick Dessein conducted all clinical assessments and prescribed appropriate treatments. I performed all data analysis with the help of my supervisors, whereafter I contributed to the interpretation of the results and writing of the manuscripts.

I hereby certify that the studies contained in this thesis have been approved by the Committee for Research in Human Subjects, University of the Witwatersrand, Johannesburg. The ethics approval numbers are M150324 and M120562 renewed as M170592.



Sulé Gunter signed on the 12th day of February 2018.



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Publications and presentations arising from this work

Publications

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Abstract

Rheumatoid arthritis (RA) increases the risk of atherosclerotic cardiovascular disease (CVD) to a similar extent as diabetes. RA is a prototypic high-grade systemic inflammation disease. In RA, systemic inflammation and its related adverse traditional cardiovascular risk factor profiles are each associated with cardiovascular event rates. The mechanisms whereby systemic inflammation enhances CVD risk in RA are currently under investigation. In this regard, impaired arterial function mediates cardiovascular events in non-RA persons. Apelin improves arterial function and reduces atherosclerosis.

This thesis comprises a series of investigations that explored the potential role of impaired arterial function and apelin in cardiovascular risk and its stratification amongst patients with RA. Associations were consistently determined in multivariate regression models.

RA patients experience arterial stiffness. We firstly assessed the relationships of comprehensively assessed traditional cardiovascular risk factors and RA disease characteristics with measures of arterial function including arterial stiffness as well as wave reflection and pressure pulsatility in 177 patients that met the American College of Rheumatology/European League Against Rheumatism classification RA criteria. Recorded characteristics explained 37% (arterial stiffness) to 71% (reflected wave pressure) of variability in arterial function. RA duration ($p=0.04$), rheumatoid factor status ($p=0.01$ to 0.03), leukocyte counts ($p=0.02$ to 0.05) and total cholesterol concentrations ($p=0.00$ to 0.03) were associated with increased central systolic pressure (cSP) and/or wave reflection markers. Body mass index ($p=0.00$ to 0.02) and insulin resistance ($p=0.00$ to 0.01) were related to reduced wave reflection and peripheral pulse pressure. Tumour necrosis factor- α inhibition ($p=0.00$) was related to reduced arterial stiffness and tetracycline use ($p=0.02$) to decreased peripheral pulse pressure (pPP). This study showed that traditional cardiovascular risk factors and RA characteristics are independently associated with measures of arterial function.

Secondly, we evaluated the potential impact of impaired arterial function on atherosclerosis in patients with RA. Carotid intima-media thickness (c-IMT) and plaque were determined by ultrasound. In 163 patients with RA, 1 SD increase in reflected wave pressure (Pb) (OR

(95%CI)=2.54 (1.41-4.44), $p=0.0001$), reflection magnitude (Rm) (OR (95%CI)=1.84 (1.17-2.89), $p=0.0008$), central pulse pressure (cPP) (OR (95%CI)=1.89 (1.12-3.22), $p=0.03$) and pPP (OR (95%CI)=2.09 (1.23-3.57), $p=0.007$) were associated with carotid plaque. The association of wave reflection with plaque was independent of arterial stiffness and pressure pulsatility, and was present in both hypertensive and normotensive RA patients. In receiver operator characteristic curve analysis, the optimal cutoff value for reflected wave pressure in predicting plaque presence was 25 mmHg with a sensitivity, specificity, positive predictive value and negative predictive value of 45.2%, 89.3%, 78.6% and 66.2%, respectively. Arterial function was not independently related to c-IMT. We concluded that consideration and therapeutic targeting of wave reflection may improve CVD prevention in RA.

Apelin causes vasodilation and reduces blood pressure but these effects are dependent on endothelial integrity. Therefore, in the third investigation, we determined the association of apelin concentrations with measures of arterial function in 170 RA patients. Apelin concentrations were not associated with arterial function in all patients. Inflammation impacted the apelin-arterial function relations (interaction $p < 0.05$). In stratified analysis, apelin levels were associated with decreased cSP (partial $r = -0.33$, $p=0.01$), cPP (partial $r = -0.26$, $p=0.04$) and forward wave pressure (partial $r = -0.33$; $p=0.01$) in patients without but not with joint deformities; apelin concentrations were related to reduced cSP in those with a Disease Activity Score in 28 joints (DAS 28) below but not ≥ 2.8 (median value) (partial $r = -0.24$, $p=0.05$) and in those with an erythrocyte sedimentation rate (ESR) below but not ≥ 13 mm/h (median value) (partial $r = -0.30$, $p=0.01$). Taken together, apelin may improve arterial function in RA but this potential effect may be lost among patients with a large inflammatory burden.

Fourthly, we evaluated the potential effects of apelin on ultrasound determined atherosclerosis and markers of plaque stability (matrix metalloproteinase (MMP)-2 and MMP-9) in 235 patients with RA. Apelin concentrations were inversely associated with those of MMP-9 (β (SE) = -0.239 (0.060), $p=0.000$). Apelin concentration-subclinical CVD relations were influenced by population origin, RA disease activity, ESR and interleukin-6 concentrations (interaction $p = 0.001$ to 0.04). Accordingly, apelin concentrations were directly associated with those of MMP-2 in white but not

black patients (β (SE) = 0.367 (0.146), $p=0.01$), in those with an ESR below but not ≥ 12 mm/h (median value) (β (SE) = 0.428 (0.143), $p=0.0003$), and in those with an interleukin-6 concentration of below but not ≥ 3.25 pg/ml (median value) (β (SE) = 0.485 (0.288), $p=0.04$); apelin levels were inversely related to cIMT in patients with RA remission or mild (β (SE) = -0.068 (0.033), $p = 0.04$) but not moderate or high disease activity (β (SE) = 0.015 (0.112), $p = 0.7$). This investigation revealed that apelin concentrations are associated with altered plaque stability mediator levels and atherosclerosis in RA. These relationships are partially dependent on population origin and systemic inflammatory status.

Taken together, the findings reported in this thesis advance our understanding of the mechanisms by which CVD risk is increased in patients with RA. Evidence is provided that disease characteristics including particularly inflammation and traditional cardiovascular risk factors are strongly associated with measures of arterial function in RA. Impaired arterial function is independently related to atherosclerosis in patients with RA. Impaired arterial function may therefore link cardiovascular risk factor exposure to CVD outcomes in patients with RA. Additionally, apelin may protect against impaired arterial function and atherosclerosis in RA. In this regard, a loss of beneficial apelin effects on the vasculature may contribute to the reported relationship between disease severity and enhanced cardiovascular risk in RA. Apelin holds promise as a therapeutic target in the present context. Our findings further reiterate the need for adequate disease control and CVD risk management in patients with RA.

Abbreviations

AAA	Abdominal aortic aneurysm
ABCA1	ATP-binding cassette transporter A
ACEI	Angiotensin converting enzyme inhibitors
ACPA	Anti-citrullinated protein antibody
Alx	Augmentation index
ARB	Angiotensin receptor blockers
BMI	Body mass index
β	Beta-value
CAD	Coronary artery disease
CDAI	Clinical Disease Activity Score
cIMT	Carotid intima-media thickness
CI	Confidence interval
CKD-EPI	Chronic Kidney Disease Epidemiology Collaboration equation
cPP	Central pulse pressure
cSP	Central systolic pressure
CVD	Cardiovascular disease
d	Diastole
DAS 28	Disease Activity Score in 28 joints
DBP	Diastolic blood pressure
DMARD	Disease-modifying antirheumatic drugs
ECG	Electrocardiogram
ELISA	Enzyme-linked immunosorbent assay
ESR	Erythrocyte sedimentation rate
EULAR	EUropean League Against Rheumatism
FSmw	Midwall fractional shortening
HAQ-DI	Stanford Health Assessment Questionnaire disability index
HDL	High density lipoprotein

HOMA-IR	Homeostatic Model Assessment of insulin resistance
HTN	Hypertension
IL-6	Interleukin-6
IQR	Interquartile range
IVST	Interventricular septum thickness
LV	Left ventricular
LVEDD	Left ventricular end diastolic diameter
LVESD	Left ventricular end systolic diameter
LVESV	Left ventricular end systolic volume
LVM	Left ventricular mass
LVMI	Left ventricular mass indexed to body height to the power 1.7
mmHg	Millimeters of mercury
MMP	Matrix metalloproteinase
m/s	Meter per second
MTX	Methotrexate
OR	Odds ratio
Pb	Reflected wave magnitude
Pf	Forward wave pressure
pg/ml	Picogram per millilitre
PPamp	Pulse pressure amplification
pPP	Peripheral pulse pressure
Pyr ¹	Pyroglutamyl
PWT	Posterior wall thickness
RA	Rheumatoid arthritis
RF	Rheumatoid factor
ROC	Receiver operator characteristic
Rm	Reflection magnitude
s	Systole

SBP	Systolic blood pressure
SCORE	Systematic COronary Risk Evaluation
SD	Standard deviation
SE	Standard error
TNF- α	Tumour necrosis alpha
PWV	Pulse wave velocity
WCC	White cell (leukocyte) count
WHR	Waist to hip ratio

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Introduction

Cardiovascular disease (CVD) is a principle cause of death worldwide (Mozaffarian *et al.*, 2016). Although known CVD risk factors including smoking, hypertension, diabetes, physical inactivity, dyslipidemia and a Western diet contribute to CVD (O'Donnell *et al.*, 2010, Lloyd-Jones *et al.*, 2010), emerging evidence suggests that these factors cannot fully account for all cardiovascular events (Menotti *et al.*, 2009; Mack *et al.*, 2014; Goff *et al.*, 2014). Beside traditional risk factors, the presence of chronic systemic inflammation is strongly implicated in CVD morbidity and mortality (Ridker *et al.*, 2000, 2003; Libby *et al.*, 2002; Mason *et al.*, 2014). High levels of circulating inflammatory cytokines may therefore also provide a pathophysiological link between diseases characterized by high levels of inflammation and the noted increase in risk of CVD.

Cardiovascular disease and risk factors in rheumatoid arthritis

It is well established that persons with rheumatoid arthritis (RA) demonstrate increases of up to 50% in cardiovascular event and mortality rates (Avina-Zubeita *et al.*, 2012; Avina-Zubeita *et al.*, 2008; Agca *et al.*, 2017), resulting in similar CVD risk rates as in the diabetic population (Van Halm *et al.*, 2009). Although certain traditional cardiovascular risk factors are more prevalent in RA (Boyer *et al.*, 2011; Chung *et al.*, 2012), conventional CVD risk stratification significantly underestimates the extent of CVD risk in RA (Dessein *et al.*, 2013^a). High-grade inflammation and genetic polymorphisms also contribute to enhanced cardiovascular risk in RA (Sattar *et al.*, 2003; Palomino-Morales *et al.*, 2010; Gonzalez-Gay *et al.*, 2008; Rodríguez-Rodríguez *et al.*, 2011; López-Mejías *et al.*, 2016). The mechanisms whereby CVD risk is increased in RA are currently undergoing elucidation (Castaneda *et al.*, 2016). In this regard, one of the possible mechanisms whereby systemic inflammation may influence cardiovascular health adversely is by impairing arterial function.

Arterial function and its relation to CVD

Aging as well as cardiovascular risk factor exposure result in arteriosclerosis, which involves in-part destruction of elastic tissue, hypertrophy and hyperplasia of smooth muscle cells and an increase in collagen deposition in the interstitium of large vessels such as the aorta (Barodka *et al.*, 2011). These changes have been associated with increases in large vessel stiffness (Dart *et al.*, 2001; Domanski *et al.*, 2002; Lemogoum *et al.*, 2004; Nair *et al.*, 2005; Vlachopoulos *et al.*, 2010^a; Vlachopoulos *et al.*, 2010^b; Wang *et al.*, 2010; Chirinos *et al.*, 2012; Cecelja *et al.*, 2012; Townsend *et al.*, 2015). In the section below I will highlight the various measures of arterial stiffness and the evidence that they relate to cardiovascular outcomes.

Pulse wave velocity (PWV) measures the rate of wave transmission and is a validated index of arterial stiffness that is considered the 'gold standard' measurement (Laurent *et al.*, 2006; Townsend *et al.*, 2015). The index of PWV relates the velocity by which the pulse wave travels within a vessel, to the distensibility of that vessel and therefore, a stiffer vessel will have a faster PWV. PWV is obtained by measuring the time taken for a pulse wave to travel a specified distance, divided by the time to attain the distance. Carotid-femoral PWV is the most frequently studied arterial function measure and has been used in several landmark studies on arterial stiffness (Mancia *et al.*, 2007; Boutouyrie *et al.*, 2015) including the Framingham Heart Study (Mitchell *et al.*, 2005).

Carotid-femoral PWV is considered to be the only accurate index of aortic stiffness (Laurent *et al.*, 2006, Boutouyrie *et al.*, 2002) and most consistently relates to cardiovascular outcomes in both the general and clinical populations, independent of confounders (Blacher *et al.*, 1999, 2003; Laurent *et al.*, 2001, Meaume *et al.*, 2001, London *et al.*, 2001, Boutouyrie *et al.*, 2002, Cruickshank *et al.*, 2002, Sutton-Tyrrell *et al.*, 2005; Mattace-Raso *et al.*, 2006, Mitchell *et al.*, 2010). In this regard, a meta-analysis of 17 longitudinal studies showed that an increase in aortic PWV of 1 m/s increases the relative risk of both all cause and cardiovascular mortality by 15% ($p < 0.001$) and independently of other risk factors (Vlachopoulos *et al.*, 2010^a).

One of the major consequences of increased arterial stiffness is that the impedance against which the heart has to pump is increased. This augments central systolic pressure. Indeed, increases in central aortic blood pressure are associated with subclinical CVD (Roman *et al.*, 2007; Subherwal *et al.*, 2010) and cardiovascular events (Safar *et al.*, 2002; Roman *et al.*, 2007; Pini *et al.*, 2008; Jankowski *et al.*, 2008; Vlachopoulos *et al.*, 2010^b; McEniery *et al.*, 2014). This increase in central aortic pressure in turn increases central pulse pressure and reduces coronary blood flow (Chae *et al.*, 1999; Dart *et al.*, 2001; Domanski *et al.*, 2002; Nair *et al.*, 2005; Townsend *et al.*, 2015; Vlachopoulos *et al.*, 2010^b). Likewise, increases in central pulse pressure have been associated with cardiovascular events (Pini *et al.*, 2008; Roman *et al.*, 2009; Vlachopoulos *et al.*, 2010^b).

Pulse pressure (SBP-DBP) is regarded as the simplest surrogate measure of arterial dysfunction. Pulse pressure provides an indication of the degree of impairment of the buffering capacity of larger arteries, and has been shown to predict CVD in both the general (Assmann *et al.*, 2005) and clinical populations (Blacher *et al.*, 2000; Safar *et al.*, 2002; Mannucci *et al.*, 2006; Nilsson *et al.*, 2009; Liu *et al.*, 2016). Two important determinants of pulse pressure are stroke volume and proximal aortic stiffness or distensibility (Dart *et al.*, 2001). Yet, although stroke volume contributes to pulse pressure, it is not regarded as a marker of aortic stiffness by all (Lemogoum *et al.*, 2004). It is also well established that central aortic pressure and central pulse pressure predict CVD risk beyond that of brachial blood pressure and brachial pulse pressure (Roman *et al.*, 2007; Pini *et al.*, 2008).

Moreover, as one moves distally along the arterial tree, the elastic properties change resulting in a progressive amplification of the pressure waveform (Wilkinson *et al.*, 2001; Laurent *et al.*, 2006; Nichols *et al.*, 2008; Avolio *et al.*, 2009; Mitchell *et al.*, 2010; Barodka *et al.*, 2011). This gives rise to a higher pulse pressure in the small to medium vessels compared to the large central arteries and is known as peripheral-to-central pulse pressure amplification. Ageing related reductions in proximal arterial compliance reduce the difference between aortic and brachial pulse pressure

leading to decreased pulse pressure amplification. Reductions in pulse pressure amplification increase cardiac workload and have been associated with adverse cardiac remodeling (Benetos *et al.*, 2010), and cardiovascular outcomes (Benetos *et al.*, 2010, Regnault *et al.*, 2012, Benetos *et al.*, 2012).

Besides the increased workload to the heart, increased aortic stiffness may also contribute to cardiovascular events by facilitating the transmission of pulsatile energy into end-organ circulatory systems (Mitchell *et al.*, 2011). Thus, although increases in arterial stiffness, as indexed from aortic PWV measurements, have been consistently associated with cardiovascular outcomes beyond cardiovascular risk factors (Blacher *et al.*, 1999, 2003; Laurent *et al.*, 2001; Meaume *et al.*, 2001; London *et al.*, 2001; Cruickshank *et al.*, 2002; Mattace-Raso *et al.*, 2006; Mitchell *et al.*, 2010), not all studies support the view that increases in aortic blood pressure or decreases in pulse pressure amplification predict outcomes beyond brachial blood pressure (Dart *et al.*, 2006, Vlachopoulos *et al.*, 2010^b, Mitchell *et al.*, 2010). Therefore, an alternative large artery change that has been hypothesized to be of importance with respect to the pathogenesis of cardiovascular events is increased wave reflection.

As pressure waves travel along the arterial tree, they are reflected off medium and small vessels back to the central aorta (Mitchell *et al.*, 2004). Therefore, the aortic pressure pulse waveform consists of the sum of the incident (forward) and reflected waves (Avolio *et al.*, 2009; Mitchell *et al.*, 2010; Barodka *et al.*, 2011). Wave reflection back to the aorta can be altered by changes in blood vessel composition and diameter, as well as branching points occurring distally. In younger persons where arterial compliance is intact, reflected waves return to the central aorta during diastole whereby they enhance diastolic perfusion pressure within the coronary circulation (Nichols & Edwards, 2001). While general vasoconstriction causes reflection points to occur more proximal to the heart, increased arterial stiffness also cause the reflected wave to travel more rapidly along the arterial tree (Nichols & Edwards, 2001; Safar *et al.*, 2003). The net result is that the faster traveling reflected wave arrives at the heart during systole, where it augments aortic systolic

pressures and reduces diastolic pressure, ultimately limiting coronary perfusion. The consequences of increased wave reflection are far reaching in that central pulse pressure is enlarged, pulse pressure amplification is reduced and the overall risk of cardiovascular events is significantly increased (Dart *et al.*, 2001). Wave reflection also returns a portion of pulsatile energy content of the waveform to the central aorta, limiting the transmission of pulsatile energy into the periphery where it might damage the microcirculation (Safar *et al.*, 2003; Mitchell *et al.*, 2011). In this regard, these effects do not require increases in central aortic pressures. The measurement of brachial blood pressure is not sensitive in determining the detrimental effects of increased wave reflection on end organ circulatory systems (Sibiya *et al.*, 2015).

Conventionally, the augmentation index (Alx) has been used as a marker of wave reflection (Wang *et al.*, 2010; Chirinos *et al.*, 2012). The Alx is calculated as the increment in pressure after the first systolic shoulder to the peak of the aortic pressure wave, which is then expressed as a percentage of the pulse pressure (Kelly *et al.*, 1989). The Alx is therefore a measure of the contribution of wave reflections to the rise in central systolic and pulse pressure (Jain *et al.*, 2014).

Although some have found a significant association between Alx and cardiovascular events (London *et al.*, 2001; Ueda *et al.*, 2004; Weber *et al.*, 2005; Chirinos *et al.*, 2005; Vlachopoulos *et al.*, 2010^b), this finding was not reproduced in all studies (Mitchell *et al.*, 2010; Hayashi *et al.*, 2014). Also, due to various confounding factors, a number of studies have argued against reliance on the Alx in various clinical settings and its use has additionally been discouraged in younger populations (Wilkinson *et al.*, 2002; Van Trijp *et al.*, 2004, 2005; Tartiere *et al.*, 2006; Hamilton *et al.*, 2007). Recently, it was recognized that augmentation pressure and hence Alx is not an accurate index of wave reflection as it incorporates a significant proportion of the forward wave. Indeed, more recent data show stronger relations between indices of wave reflection determined using wave separation analysis (which segregates the forward and reflected waves) and cardiovascular outcomes (Wang *et al.*, 2010; Chirinos *et al.*, 2012).

The measurement of the absolute reflected wave pressure using wave separation analysis is independent of reflected wave transit time and has indeed been identified as a better measure of wave reflection intensity than Alx (Segers *et al.*, 2007; Wang *et al.*, 2010). Hence, the impact of wave reflection on central pulse pressure (Mitchell *et al.*, 2008), central systolic pressure (Hope *et al.*, 2005), target organ damages and cardiovascular events may have been underestimated when the Alx was used (Wang *et al.*, 2010). Several large population based studies have confirmed that, when using wave separation analysis, the reflected wave is independently predictive of long-term cardiovascular mortality and target organ changes (Wang *et al.*, 2010, Chirinos *et al.*, 2012; Booyesen *et al.*, 2015; Phan *et al.*, 2016). The impact of wave reflection on CVD risk is thereby becoming increasingly recognized (Townsend *et al.*, 2015). Notably, these studies found the reflected wave to be predictive of cardiovascular events, independent of arterial stiffness (Wang *et al.*, 2010) or brachial systolic, diastolic, and mean blood pressures (Weber *et al.*, 2012).

In addition to the reflected wave, the forward wave as a marker of proximal arterial function and peak aortic flow (Mitchell *et al.*, 2008; 2015; Torjesen *et al.*, 2014; Cooper *et al.*, 2015) has shown to contribute to elevated pulse pressure with ageing, whereas the reflected wave contributes only modestly to the respective increase (Mitchell *et al.*, 2010; Fok *et al.*, 2014; Torjesen *et al.*, 2014; Cooper *et al.*, 2015). It is also believed that the reflected wave is dependent on the forward wave. Moreover, the reflection magnitude, expressed as the ratio of forward and backward traveling components, was found to be strongly and independently predictive of all cardiovascular events (Chirinos *et al.*, 2012).

Taken together, arterial dysfunction increases the risk of cardiovascular events. Thus, chronic inflammation could contribute towards cardiovascular events through increases in either large artery (aortic) stiffness or by enhancing aortic wave reflection. Additionally, either of these changes can mediate deleterious effects by increasing aortic blood pressure. These alterations are not detected at the brachial artery and are associated with decreased pulse pressure

amplification. What is the evidence to show an impact of chronic inflammation on large artery stiffness, reflective wave function, aortic blood pressure or pulse pressure amplification?

Researchers have utilized chronic high-grade inflammatory conditions such as RA as models to investigate the effects of inflammation on arterial function. Chronic systemic inflammation causes vascular smooth muscle cell proliferation, as well as destruction of elastin fibers with a concomitant increase in collagen deposition within the aorta and other large arteries (Hattori *et al.*, 2003; Hirsch *et al.*, 2014). As previously mentioned, these vascular remodeling processes promote increases in arterial stiffness. Several studies, including a recent meta analysis, have documented that PWV (Mäki-Petäjä *et al.*, 2006; Arosio *et al.*, 2007; Pieringer *et al.*, 2009; Bazzichi *et al.*, 2009; Kim *et al.*, 2012; Turkyilmaz *et al.*, 2013; Lo Gullo *et al.*, 2014, 2015; Ambrosino *et al.*, 2015), central systolic pressure (Klocke *et al.*, 2003) and Alx (Klocke *et al.*, 2003; Avalos *et al.*, 2007; Wällberg-Jonsson *et al.*, 2008; Čypienė *et al.* 2010; Pieringer *et al.*, 2012; Lo Gullo *et al.*, 2014; Ambrosino *et al.*, 2015) are significantly increased in RA patients, when compared to controls. However, there are presently no studies that have evaluated the impact of RA on wave reflection as determined using wave separation analysis.

Although, as discussed in the above, a number of studies have demonstrated relationships between indices of aortic function and RA, the question that arises is whether these relationships can be attributed to chronic inflammation or to RA co-morbidities including metabolic abnormalities and the adverse effects of drug therapy? In regard to traditional cardiovascular risk factors, age and blood pressure are certainly the strongest determinants of aortic function (Safar *et al.*, 2003; Mitchell *et al.*, 2004). Indeed, two recent RA studies reported that after controlling for traditional cardiovascular risk factors, PWV was not increased compared to controls (Fan *et al.*, 2014; Arida *et al.*, 2015). The authors further reported that traditional cardiovascular risk factors, rather than inflammatory markers predict arterial stiffness in RA. Although age and blood pressure most strongly predict pulse wave velocity, numerous other population characteristics may further impact on arterial function. In this regard, studies have reported that besides age and blood pressure

kidney function (Wang *et al.*, 2005; Ohya *et al.*, 2006), hypothyroidism (Nagasaki *et al.*, 2006), metabolic syndrome components (Kim *et al.*, 2006), antioxidant status (Nakamura *et al.*, 2006), smoking (Lemogoum *et al.*, 2006), excessive coffee consumption (Vlachopoulos *et al.*, 2005) and mental stress (Vlachopoulos *et al.*, 2006) can all impact on arterial stiffness and thus should not be overlooked. Nevertheless arterial stiffness was recently reported to predict incident cardiovascular events in patients with RA (Ik Dahl *et al.*, 2016). The relative impact of traditional cardiovascular risk factors and disease characteristics on comprehensively evaluated arterial function thus needs further exploration.

In this thesis, we hypothesized that RA characteristics and traditional CVD risk factor exposure can each significantly affect measures of arterial function, including PWV, wave reflection and pressure pulsatility.

Association between arterial function and atherosclerosis

It is well established that persons with RA demonstrate an increased prevalence of atherosclerotic CVD (Avina-Zubeita *et al.*, 2008; Agca *et al.*, 2017). For this reason, the European League Against Rheumatism (EULAR) have recommended to use a multiplier of 1.5 when calculating the modified Systematic COronary Risk Evaluation (mSCORE) for all patients with RA, in order to more accurately predict CVD risk in this population (Agca *et al.*, 2017). EULAR also recently recommended the additional use of carotid ultrasound screening for plaque in patients with RA. This was proven to be useful in reclassifying a large proportion of RA patients into an appropriately higher CVD risk group (Corrales *et al.*, 2014). The subclinical atherosclerotic burden may have been markedly underestimated in the RA population until recently. Nevertheless, the increased rates of cardiovascular mortality in RA are not fully accounted for by either systemic vasculitis or classical cardiovascular risk factors (Del Rincón *et al.*, 2001; Van Doornum *et al.*, 2002). Whereas arterial stiffness induces CVD by means of increasing central pressures and facilitating the transmission of pulsatile energy into end-organ circulatory systems, it may additionally contribute

to the atherosclerotic process. What then is the evidence that arterial stiffness increases atherosclerosis?

Frequently employed measures used to define atherosclerosis include ultrasound determined carotid intima-media thickness (cIMT) and plaque presence. Although both these measures are well-validated markers of cardiovascular risk, they comprise phenotypes that can be differentiated both on a biological and genetic basis (Spence *et al.*, 2004; Riccio *et al.*, 2006; Johnsen *et al.*, 2009), and represent different stages of atherosclerosis progression. Intima-media thickening occurs mostly due to proliferation within the tunica media, as a result of aging and particularly hypertension (Riccio *et al.*, 2006). Intima-medial thickening is also significantly associated with risk factors for stroke as well as the incidence of stroke events (Spence *et al.*, 2004; Johnsen *et al.*, 2009). Contrary to this, carotid plaque originates in vascular pathological processes occurring within the arterial intimal lining (Simon *et al.*, 2010), and reflects more advanced atherosclerosis that is associated with coronary artery disease (CAD) risk factors and prevalence (Spence *et al.*, 2004; Riccio *et al.*, 2006; Dessein *et al.*, 2014).

Studies investigating the association between arterial stiffness and cIMT in the general population have however produced conflicting results (Cecelja *et al.*, 2012). Positive associations between PWV and cIMT (Van Popele *et al.*, 2001) as well as reflected wave and cIMT (Sibiya *et al.*, 2015) have been reported. In contrast, two other large studies (n=564 and n=605, respectively) found no associations between IMT and measures of arterial distensibility (Zureik *et al.*, 2002; Juo *et al.*, 2005). Positive associations were reported more consistently between PWV and aortic calcification (London *et al.*, 2003; Odink *et al.*, 2008; McEniery *et al.*, 2009), as well as with the total plaque burden in the carotid artery (Van Popele *et al.*, 2001). However, further studies are required to confirm these findings. Apart from age and blood pressure, traditional cardiovascular risk factors correlate weakly with arterial stiffness. Alternative mechanisms are likely responsible for associations between arterial stiffness and atherosclerosis.

In this regard, chronic systemic inflammation is strongly implicated in RA, as it is associated with the development and progression of atherosclerosis (Dessein *et al.*, 2005). Systemic inflammation also causes destabilization of former stable plaques, as well as superimposed thrombosis that typically precipitates acute vascular events (Van Doornum *et al.*, 2002; Libby *et al.*, 2006). Similarly, as discussed above, inflammation was shown to be associated with arterial stiffness. More specifically, systemic inflammation causes endothelial activation and dysfunction, which is implicated in the development of both arterial stiffness and atherosclerosis in RA (Dessein *et al.*, 2005; Dessein & Joffe, 2006; Södergren *et al.*, 2010; Dessein *et al.*, 2013^b). Traditional cardiovascular risk factors may also exert their detrimental effects on arterial stiffness and atherosclerosis by inducing endothelial dysfunction (Oliver & Webb, 2003).

However, the evidence that arterial dysfunction is associated with atherosclerosis in RA, and the role of traditional risk factors and inflammation in this process are controversial. One study found a marginal association between Alx and coronary artery calcification (Avalos *et al.*, 2007), while a more recent study concluded that neither PWV nor Alx were independently associated with carotid atherosclerosis (Robustillo-Villarino *et al.*, 2017). In addition, the association between wave reflection as assessed by wave separation analysis and atherosclerosis in RA patients, has not been investigated so far. As wave reflection depends on blood vessel composition and diameter, and particularly occurs at different arterial locations due to vessel branching, bending and bifurcating, it may be more closely related to atherosclerotic lesions than other arterial properties. Indeed, atherosclerosis also develops predominantly at branches, bends, and bifurcations within the arteries as disturbed or reduced blood flow imparts lower or oscillatory shear stress on the vessel wall (Zarins *et al.*, 1983; Warboys *et al.*, 2011). In contrast, other areas experiencing higher shear stress and unidirectional blood flow are protected from lesion development. The relationship between comprehensively assessed arterial function and atherosclerosis in RA requires further investigation.

We therefore hypothesized that reflected wave pressure as determined in separation analysis is associated with atherosclerosis in RA. Additionally, we aimed to confirm the previously reported finding that conventional measures of arterial function including Alx and PWV, are not associated with atherosclerosis in patients with RA (Robustillo-Villarino et al., 2017).

Apelin

The endogenous peptide apelin has received much attention recently, particularly in view of its beneficial effects on the cardiovascular system. Apelin acts on the APJ receptor, which is classified as a 7-transmembrane G-protein coupled receptor (O'Carroll *et al.*, 2013). The APJ receptor was identified in 1993 by O'Dowd and colleagues, and remained an orphan receptor until 1998 when Tatemoto and others identified its endogenous ligand, apelin-36, from isolated bovine stomach extracts. Apelin-36 originates from the C-terminal portion of the 77-aminoacid pre-protein. Apelin-36 is cleaved into short peptides with 13 to 19 aminoacids, which exhibit significantly higher activity than apelin-36 (Tatemoto *et al.*, 1998; Kawamata *et al.*, 2001). More specifically, the apelin-13 isoform demonstrates the greatest capacity to regulate the cardiovascular system (Folino *et al.*, 2015).

Apelin and APJ mRNA are widely expressed in peripheral bodily tissues, including the cerebellum, lungs, kidneys, and especially peripheral adipocytes and endothelial cells (Boucher *et al.*, 2005). There are also notably high concentrations observed in the cardiovascular system (Kawamata *et al.*, 2001; Lee *et al.*, 2000; Hosoya *et al.*, 2000; Barnes *et al.*, 2010; O'Carroll *et al.*, 2000). APJ receptors are expressed significantly in various cell types within the heart, including the endothelium, smooth muscle cells and myocytes (Kleinz *et al.*, 2005). Also, apelin was shown to be produced in the endothelial linings to a significantly greater extent compared to cardiomyocytes and vascular smooth muscle cells (Kleinz *et al.*, 2004). Endothelial apelin thereby plays a role as a locally secreted cardiovascular mediator acting on APJ receptors to regulate vascular tone and cardiac contractility.

The role of apelin in the cardiovascular system

The therapeutic potential of the apelin/APJ pathway is evident when observing its various beneficial effects on vascular and cardiac function (Ashley *et al.*, 2005). Firstly, apelin has been shown to produce a potent inotropic effect on animal preparations (Szokodi *et al.*, 2002; Berry *et al.*, 2004; Ashley *et al.*, 2005). Chronic administration of apelin increased the velocity of circumferential shortening and increased cardiac output, without causing hypertrophy (Ashley *et al.*, 2005). Apelin effectively reduces left ventricular preload and afterload and increased contractile reserve (Ashley *et al.*, 2005). The inotropy induced by apelin is thought to be brought about by enhancing myofilament sensitivity to calcium (Farkasfalvi *et al.*, 2007; Perjés *et al.*, 2014).

Secondly, apelin has direct beneficial effects on the vasculature, which has been the subject of recent research. In vitro experiments have demonstrated how apelin can act as either a vasodilator or vasoconstrictor. In isolated rat aortas, the mechanism of vasodilation was shown to involve the L-arginine/nitric oxide synthase pathway, whereby nitric oxide synthase expression and sensitivity was effectively increased resulting in vasodilation and associated reductions in blood pressure (Jia *et al.*, 2007; Japp *et al.*, 2010). These findings were also confirmed in humans (Japp *et al.*, 2008^a). Apelin additionally antagonizes vasoconstriction brought about by angiotensinogen/angiotensin II pathway (Chun *et al.*, 2008). Importantly however, the vasodilatory effects of apelin depend on endothelial integrity (Tatemoto *et al.*, 2001; Ishida *et al.*, 2004; Japp *et al.*, 2008^a; Han *et al.*, 2013). In this regard, Hashimoto and colleagues (2006) demonstrated that following endothelial injury, apelin crosses the endothelial border and binds to receptors in vascular smooth muscle cells, where it induces phosphorylation of the myosin light chains. These findings were confirmed in humans where apelin was shown to induce vasoconstriction in a saphenous vein denuded of a functional endothelium (Katugampola *et al.*, 2002). This suggests that the primary vasodilatory effects of apelin are mediated by nitric oxide production from an intact endothelium. Although the positive inotropic and vasodilatory effects of apelin have been well established, further evidence is required to clarify its role in the pathogenesis of CVD.

Apelin and the pathogenesis of CVD

Apelin is believed to play a cardioprotective role in various CVD conditions. In this regard, apelin is reported to be reduced in patients with hypertension and left ventricular dysfunction (Przewlocke-Kosmala *et al.*, 2011), heart failure (Iwanaga *et al.*, 2006; Chong *et al.*, 2006; Japp *et al.*, 2008^b; Gao *et al.*, 2009) or acute myocardial infarction (Weir *et al.*, 2009; Kadoglou *et al.*, 2010; Grzywocz *et al.*, 2015). It is believed that upregulation of apelin/APJ concentration after myocardial infarction improves coronary blood flow and maintains contractile capacity (Yamaleyeva *et al.*, 2016) and that it preserves cardiac regenerative capacity (Li *et al.*, 2013; Zhang *et al.*, 2016).

An intrinsic part of the cardioprotective function of apelin revolves around its ability to suppress the production of certain cytokines (tumour necrosis factor- α and interleukin 1- β) as well as impeding excessive collagen production (Leeper *et al.*, 2009; Koguchi *et al.*, 2012; Pchejetski *et al.*, 2012). As RA is characterized by high concentrations of circulating cytokine levels, and collagen production is implicated in arteriosclerosis and atherosclerosis, the role of apelin in CVD progression in RA merits investigation.

Circulating apelin concentrations are significantly decreased in RA (Di Franco *et al.*, 2012). Patients with RA commonly demonstrate premature endothelial activation (Södergren *et al.*, 2010; Dessein & Joffe, 2006) and newly diagnosed patients often have vascular dysfunction (Bergholm *et al.*, 2002). Increased inflammatory status is related to endothelial dysfunction in RA, independent of other non-traditional and traditional cardiovascular risk factors (Dessein *et al.*, 2005; Dessein *et al.*, 2013^b). Importantly, increased inflammatory activity results in reduced nitric oxide production and more pronounced blunting of endothelium and nitric oxide-dependent vasodilatation (Bergholm *et al.*, 2002; Yki-Järvinen *et al.*, 2003). Therefore, inflammation induced endothelial dysfunction may predispose persons with RA to CVD (Bergholm *et al.*, 2002). However, what is the evidence that apelin may improve CVD risk profiles by enhancing vascular tone?

Early studies in diabetic mice demonstrated beneficial apelin vascular effects that comprised a reversal of endothelial dysfunction (Zhong *et al.*, 2007). Furthermore, an 8-week aerobic exercise training intervention resulted in significant increases in plasma apelin concentrations, which correlated with reductions in arterial stiffness (Fujie *et al.*, 2014). Genetic polymorphisms of the apelinergic system components are associated with earlier onset of hypertension (Li *et al.*, 2009), as well as increases in arterial stiffness (Liao *et al.*, 2015). Reported evidence thus suggests that apelin exerts cardioprotective effects, which are brought about by significant improvements in arterial function. However, whether apelin protects against arterial dysfunction in RA remains unknown.

The third hypothesis in this thesis is therefore that apelin concentrations are independently associated with measures of arterial function in RA. However, given that cytokine concentrations are capable of altering vascular tone, induce endothelial dysfunction and reduce apelin concentrations, we further hypothesized that these associations between apelin and arterial function depend on inflammatory status in RA.

Besides its role in arterial stiffness, endothelial dysfunction also initiates and contributes to the progression of atherosclerosis (Ik Dahl *et al.*, 2015). In this regard, apelin is thought to have potent antiatherogenic properties. Apelin improves vascular function via a reduction in oxidative stress, and by promoting the release of endothelial nitric oxide, a free radical scavenger (Liu *et al.*, 2013). Apelin also reduces inflammation and enhances the expression of adherence factors (Sorli *et al.*, 2006; Simpkin *et al.*, 2007; Fukushima *et al.*, 2010), thereby reducing the progression of atherosclerosis. Chun and colleagues (2008) made use of apolipoprotein E null murine models to demonstrate that apelin counteracts the angiotensin II mediated increase in atherosclerotic disease burden and prevents adverse vascular remodeling and aneurysm formation in mice, independent of blood pressure or a high cholesterol diet (Chun *et al.*, 2008). Therefore, the action of apelin may be essential in combating atherosclerosis and maintaining optimal vascular health.

The anti-atherogenic function of apelin is further thought to be mediated through activation of protein kinase C, which phosphorylates and stabilizes ATP-binding cassette transporter A1 (ABCA1) and inhibits calpain-mediated degradation of ABCA1 (Liu *et al.*, 2013). ATP-binding cassettes are responsible for reverse cholesterol transport. Accordingly, apelin markedly increases cholesterol efflux from macrophages, preventing foam cell formation (Liu *et al.*, 2013).

Patients with hypercholesterolemia have decreased plasma apelin concentrations when compared to controls (Tasci *et al.*, 2007). Likewise, apelin levels are reportedly decreased in patients with CAD (Akçilar *et al.*, 2015). Apelin levels were lower in more severe CAD patients, and were associated with acute phase CAD, independent of other cardiovascular risk factors (Kadoglou *et al.*, 2010). This suggests a role for apelin in the progression and destabilization of coronary artery plaques (Kadoglou *et al.*, 2010). In this regard, following statin therapy, which promotes reverse cholesterol transport, both apelin concentrations and plaque stability markers were significantly increased (Kadoglou *et al.*, 2012).

Moreover, an indirect association between apelin and plaque stability and vulnerability mediators, specifically matrix metalloproteinases (MMPs), has been previously reported (Webb *et al.*, 2007; Leeper *et al.*, 2009;). In this regard, MMPs represent a family of 28 zinc-dependent endopeptidases, which are involved in the metabolism of extracellular matrix proteins, including collagen and elastin. The proteinase activity exerted by 11 of the 28 MMPs have been implicated in the development and progression of atherosclerosis, as well as ischemic insults occurring as a result of atherosclerotic plaque rupture. Importantly, MMPs have counteracting roles in the modulation of atherogenesis, vascular remodeling and plaque rupture. MMPs may either enhance fibrosis and intimal thickening, resulting in more stable plaques, or encourage extracellular matrix degradation, resulting in increased plaque vulnerability and rupture (Bäck *et al.*, 2010; Newby *et al.*, 2015). MMPs are prone to activation by inflammatory signaling (Reel *et al.*, 2011; Huang *et al.*, 2012), including that of angiotensin II (Lakatta *et al.*, 2013; Wang *et al.*, 2014; Wang *et al.*, 2015). In this regard, there is a definite potential for the apelin/APJ axis in providing protection against

atherosclerosis and atherosclerotic disease progression (Barnes *et al.*, 2010). Furthermore, apelin may be of special interest to patients with RA, in whom the increased atherosclerotic burden is well established.

We therefore hypothesized that apelin concentrations are independently associated with ultrasound determined atherosclerosis as measured by cIMT and the presence of plaque in RA. We further hypothesized that apelin concentrations are independently associated with concentrations of MMPs as markers of plaque stability and vulnerability in RA.

Cardiovascular Risk Factors and Disease Characteristics Are Consistently Associated with Arterial Function in Rheumatoid Arthritis

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ABSTRACT. Objective. Arterial properties influence cardiovascular disease (CVD) risk. We identified potential determinants of arterial function in patients with rheumatoid arthritis (RA).

Methods. Relationships of traditional cardiovascular risk factors and RA characteristics with arterial stiffness (pulse wave velocity), wave reflection (augmentation index, reflected wave pressure, and reflection magnitude), and pressure pulsatility (central systolic and pulse pressure, peripheral pulse pressure, pulse pressure amplification, and forward wave pressure) were identified in multivariable backward regression models among 177 patients without established CVD (118 white, 32 Asian, 22 black, 5 mixed ancestry).

Results. Recorded characteristics explained 37% (pulse wave velocity) to 71% (reflected wave pressure) of the variability in arterial function. These factors were particularly associated with wave reflection and pressure pulsatility: RA duration ($p = 0.04$), rheumatoid factor status ($p = 0.01$ to 0.03), leukocyte counts ($p = 0.02$ to 0.05), and total cholesterol ($p < 0.01$ to 0.03). Body mass index ($p < 0.01$ to 0.02) and insulin resistance ($p < 0.01$ to 0.01) were related to reduced wave reflection and peripheral pulse pressure. Exercise ($p = 0.02$) and alcohol consumption ($p < 0.01$) were associated with increased pulse pressure amplification and decreased peripheral pulse pressure, respectively. Tumor necrosis factor- α inhibition ($p < 0.01$) was related to reduced pulse wave velocity, and tetra-cycline use ($p = 0.02$) to decreased peripheral pulse pressure.

Conclusion. Traditional cardiovascular risk factors and disease characteristics are consistently associated with vascular hemodynamic alterations in RA. The relative effect of arterial stiffness, wave reflection, and pressure pulsatility on CVD risk in RA needs further study. (First Release June 1 2017; J Rheumatol 2017;44:1125–33; doi:10.3899/jrheum.170029)

Key Indexing Terms:
RHEUMATOID ARTHRITIS
WAVE REFLECTION

ARTERIAL STIFFNESS
PRESSURE PULSATILITY

Aging causes aortic elastin fibers to be replaced by collagen^{1,2}. This process results in arteriosclerosis, which is increased by exposure to cardiovascular risk factors but can occur in the absence of atherosclerosis^{1,2}. Arteriosclerosis causes arterial stiffness that is most frequently evaluated by pulse wave velocity. Pulse wave velocity represents the velocity at which the pressure wave propagates through

arteries. Arterial stiffness increases central systolic blood pressure (BP) and cardiac workload.

The peripheral arterial wall contains mostly muscle fibers and is therefore stiffer than the aorta^{1,2,3,4,5}. Consequently, in healthy young persons, the peripheral pulse pressure is substantially larger than that in the aorta^{1,2,3,4,5}. This is referred to as (peripheral-to-central) pulse pressure amplifi-

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cation, which decreases with vascular aging. The aortic pressure wave comprises the sum of the forward and reflected wave^{1,2,3,4,5}. Wave reflection back to the aorta is mediated by changes in blood vessel wall composition and branching points distally along the arterial tree. In normal health, the reflected wave arrives at the heart during early diastole and aids coronary blood flow. Arterial stiffness increases wave reflection. Consequently, the reflected wave returns faster centrally and arrives at the heart during systole. Wave reflection thereby augments central BP and cardiac workload, and reduces diastolic BP and coronary perfusion. Increased wave reflection contributes to increased pulse pressure and reduced pulse pressure amplification.

The adverse effects of wave reflection on end-organ circulatory systems cannot be detected by brachial BP measurements⁶. Wave reflection has been mostly estimated by the aortic augmentation index^{7,8,9}. However, the augmentation index incorporates a large proportion of the forward wave along with the reflected wave because of their overlap in time^{7,8,9,10}. In 2015, the American Heart Association recommended the use of wave separation analysis rather than augmentation index in investigations focused on the role of wave reflection as an exposure for a cardiovascular outcome or a target for intervention¹⁰. Upon using this approach, the adverse effect of wave reflection on cardiovascular disease (CVD) risk has been increasingly recognized^{7,8,9,10,11}. Enhanced wave reflection is a novel mechanical biomarker for heart failure⁸.

Arterial stiffness as assessed by increased pulse wave velocity augments the risk of CVD independent of major traditional risk factors in non-RA persons⁵. Ikdahl and colleagues recently reported that pulse wave velocity but not augmentation index is also associated with incident cardiovascular events in RA¹². In 2010, Vlachopoulos and colleagues reported that a 1 SD increase in pulse wave velocity was associated with a 47%, 47%, and 42% enhanced risk of cardiovascular events, mortality, and all-cause mortality, respectively¹³. However, among arterial function markers, not only pulse wave velocity but also a long list of factors are each independently associated with CVD risk in the general population^{1,2,3,4,5,6,7,8,9,10-17}; wave reflection assessed by the augmentation index, reflected wave pressure, and reflection magnitude defined as the reflected-forward wave pressure ratio, as well as pressure pulsatility evaluated by central systolic and pulse pressure, peripheral pulse pressure, pulse pressure augmentation, and forward wave pressure¹⁴. The latter hemodynamic measures are the result of several different factors, including but not limited to arterial stiffness¹⁰.

Rheumatoid arthritis (RA) increases cardiovascular event and mortality rates by about 50%^{18,19}. Disease characteristics including particularly high-grade inflammation, unfavorable traditional cardiovascular risk factor profiles, and genetic factors associate with CVD in RA²⁰. The mechanisms

involved in the increased cardiovascular risk in patients with RA are currently under investigation²⁰. In this regard, a metaanalysis of 25 studies by Ambrosino and colleagues²¹ documented a significant increase in pulse wave analysis and augmentation index in patients with RA. The aim of the present study was to identify potential determinants of comprehensively assessed arterial function in a relatively large group of ethnically diverse patients with RA.

MATERIALS AND METHODS

Patients. We initially enrolled 186 consecutive patients who met the 1987 American College of Rheumatology (ACR) and 2010 ACR/European League Against Rheumatism criteria for RA^{22,23} at the Milpark Hospital, Johannesburg, South Africa. Nine had established CVD and were excluded. The remaining 177 patients comprised 118 white, 32 Asian, 22 black, and 5 mixed ancestry participants. This study was performed in line with the principles of the Helsinki declaration and approval was obtained from the University of Witwatersrand Human (Medical) Research Ethics Committee (approval number: M06-07-33; protocol number: M120562) in Johannesburg, South Africa. All participants gave written informed consent.

Baseline characteristics. Baseline characteristics including cardiovascular risk factors were recorded using previously reported methods²⁴ and are given in the Supplementary File, available from the authors on request.

Arterial function. Central arterial function measurements were evaluated using radial applanation tonometry and SphygmoCor software, as previously reported⁹. Pulse wave velocity, central aortic BP, and its determinants (reflected and forward wave pressures) were measured using a high-fidelity SPC-301 micromanometer (Millar Instrument Inc.), interfaced with a computer using SphygmoCor software version 9.0 (AtCor Medical Pty. Ltd.). After resting for 15 min in the supine position, arterial waveforms at the radial (dominant arm), carotid, and femoral artery pulses were recorded for a period of 10 consecutive waveforms (heartbeats). The pulse wave was calibrated by manual measurement (auscultation) of brachial BP taken immediately before the recordings. Using a validated generalized transfer function incorporated in the SphygmoCor software, the peripheral pressure wave form was converted into a central aortic waveform. When systolic or diastolic variability of consecutive waveforms exceeded 5% or the amplitude of the pulse wave signal was < 80 mV, the recorded results were discarded. The aortic pulse wave velocity was calculated as distance in meters divided by transit time in seconds. The magnitude of the forward and reflected wave components of the aortic pressure waveform was determined by the SphygmoCor software that separates the aortic waveform by using a modified triangular waveform. Augmentation index was calculated as (second systolic peak/first systolic peak) × 100, reflection magnitude as (reflected wave amplitude/forward wave amplitude) × 100, and pulse pressure amplification as radial pulse pressure/aortic pulse pressure. All measurements were made by a single experienced technician who was unaware of the cardiovascular risk factor profiles of the patients. Brachial BP was recorded in all patients. Technically sound measurements of the central pressure wave and pulse wave velocity were obtained in 169 and 160 patients, respectively. Further details on arterial function measurements are given in the Supplementary File, available from the authors on request.

Data analysis. Results are presented as mean (SD), median [interquartile range (IQR)], or proportions as appropriate. Non-normally distributed characteristics were logarithmically transformed prior to statistical analysis.

Mean values of arterial function measures among patients from different population groups were compared by the ANOVA test. Correlations among arterial function measures were assessed by the Pearson's correlation coefficient test.

Age, sex, race, heart rate, body height, and brachial BP are major confounders in the present context. We assessed the association of traditional risk factors and RA characteristics with arterial function in multivariable linear regression models with adjustment for the respective features. This

revealed a large number of patient characteristics that were associated with arterial function. These characteristics were therefore consistently entered in stepwise backward regression models to identify independent relationships.

Statistical computations were made using SPSS software, version 21 (SPSS). Significance was set at $p < 0.05$.

RESULTS

The recorded patient characteristics are given in Table 1. Mean (SD) age at time of the study was 58.0 years (12.1) and median (IQR) RA duration 14.5 years (9.0–21.7). Dyslipidemia, hypertension (HTN), current smoking, and diabetes were recorded in 47.5%, 40.7%, 10.2%, and 5.6% of the patients, respectively. Lipid-lowering medications and antihypertensive agents were each used in 40% of the study population.

Rheumatoid factor (RF) and anticitrullinated protein antibody positivity were present in 75.1% and 69.5% of the participants, respectively. Disease activity was overall well controlled with a median (IQR) Clinical Disease Activity Index (CDAI) score of 5 (1–13) and a mean (SD) 28-joint Disease Activity Score (DAS28) of 2.8 (1.7); 67.8% and 61.7% of patients had mild disease activity or experienced remission according to recommended CDAI and DAS28 score cutoff values, respectively. One or more synthetic disease-modifying agents were taken by 90.3% of participants, whereas 11.3% took biologic agents. The mean (SD) central systolic pressure was 126 (16) mmHg and median (IQR) pulse wave velocity was 8.0 (6.1–9.3) m/sec.

Table 1. Recorded characteristics in 177 patients with rheumatoid arthritis. Dichotomous variables are expressed as proportions or percentages and continuous variables as mean (SD) or median (interquartile range).

Variables	Values	Variables	Values
Demographic characteristics		RF-positive	75.1
Age at study time, yrs	58.0 (12.1)	ACPA-positive	69.5
Age at disease onset, yrs	42.1 (13.8)	Clinical Disease Activity Index	5 (1–13)
Female sex	82.5	Disease Activity Score in 28 joints	2.8 (1.7)
White	66.7	ESR, mm/h	12 (4–26)
Asian	18.1	C-reactive protein, mg/l	3.2 (1.2–7.9)
Black	12.4	Leukocytes, n/nl	5.5 (4.5–7.0)
Mixed	2.8	Deformed joints, n	0 (0–10)
Lifestyle factors		Extraarticular manifestations	22
Exercise	32.2	Stanford HAQ-DI	0.375 (0.000–0.875)
Alcohol use	31.6	Synthetic disease-modifying agents	
Current smoking	10.2	Methotrexate	75.7
Anthropometry		Chloroquine	50.3
Body mass index, kg/m ²	26.8 (5.5)	Leflunomide	40.1
Waist circumference, cm	92 (14)	Sulfasalazine	16.4
Waist-to-hip ratio	0.88 (0.09)	Tetracycline	15.3
Metabolic risk factors		Azathioprine	6.8
Hypertension	40.7	Current DMARD, n	2.1 (1.1)
Systolic BP, mmHg	128 (15)	Biological disease-modifying agents	
Diastolic BP, mmHg	81 (8)	TNF- α inhibitors	8.5
Total cholesterol, mmol/l	4.5 (1.0)	Abatacept	2.8
HDL cholesterol, mmol/l	1.66 (0.46)	Nonsteroidal antiinflammatory drugs	34.5
LDL cholesterol, mmol/l	2.4 (0.9)	Prednisone use	2.3
Triglycerides, mmol/l	1.0 (0.7–1.3)	CKD-EPI, ml/min/1.73 m ²	99.3 (87.3–107.6)
Cholesterol–HDL cholesterol ratio	2.7 (2.3–3.2)	Heart rate, bpm	72 (12)
Cholesterol–HDL cholesterol ratio > 4, %	9.0	Framingham score	2.9 (5.4)
Dyslipidemia	47.5	Arterial function	
Diabetes	5.6	Central systolic BP, mmHg	126 (16)
Glucose, mmol/l	4.9 (4.5–5.1)	Central diastolic BP, mmHg	84 (9)
HOMA-IR	1.4 (1.0–1.9)	Central pulse pressure, mmHg	42 (14)
Cardiovascular agents		Aortic-femoral pulse wave velocity, m/sec	8.0 (6.1–9.3)
Antihypertensives	40.1	Aortic augmentation index, %	31 (11)
Statins	40.1	Forward wave pressure, mmHg	28 (9)
Ezetimibe	10.7	Reflected wave pressure, mmHg	21 (8)
Oral glucose-lowering agents	2.3	Reflection magnitude, %	75 (23)
Insulin	1.7	Pulse pressure amplification	1.22 (1.16–1.31)
RA characteristics		Peripheral pulse pressure, mmHg	47 (13)
RA duration, yrs	14.5 (9.0–21.7)		

HDL: high-density lipoprotein; LDL: low-density lipoprotein; BP: blood pressure; HOMA-IR: homeostatic model assessment of insulin resistance; RA: rheumatoid arthritis; RF: rheumatoid factor; ACPA: anticitrullinated protein antibodies; ESR: erythrocyte sedimentation rate; Stanford HAQ-DI: Stanford Health Assessment Questionnaire–Disability Index; DMARD: disease-modifying antirheumatic drugs; TNF: tumor necrosis factor; CKD-EPI: Chronic Kidney Disease Epidemiology Collaboration.

Disparities in arterial function measures among patients with RA with a different population origin are given in Table 2. Significant differences in mean values (logarithmic transformation was applied for data with non-normal distribution) across groups were observed for central pulse pressure and the forward and reflected wave pressure.

Table 3 shows the bivariate relations between the evaluated arterial function measures. Reflected wave pressure, central systolic pressure, and central and peripheral pulse pressure were each related to all other measurements. Pulse wave velocity was associated with other measurements except for the augmentation index and reflection magnitude, and a borderline significant relationship ($p = 0.06$) to pulse pressure amplification.

Associations of patient characteristics with arterial function. Table 4 shows the age, sex, race, heart rate, body height, and systolic and diastolic adjusted significant associations of other patient characteristics with arterial function; apart from race, these potential confounders were indeed each associated with 1 to 5 of the evaluated arterial function measures as reported in non-RA populations^{1,2,3,4,5,6,7,8,9,10–14}.

The analysis revealed that a further 20 patient character-

istics were, to a variable extent, significantly related to arterial function. These features were lifestyle factors (exercise and alcohol consumption), anthropometric measures [body mass index (BMI), waist circumference and waist-to-hip ratio], metabolic risk factors [total and high-density lipoprotein (HDL) cholesterol concentrations and homeostatic model assessment for insulin resistance (HOMA-IR)], cardiovascular drug use (statins and insulin), Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) estimated glomerular filtration rate, RA characteristics [disease duration, deformed joint count, RF status, C-reactive protein (CRP) levels and leukocyte count], and RA treatment with methotrexate (MTX), chloroquine, tetracycline, and tumor necrosis factor (TNF)- α inhibition. Among anthropometric measures, BMI was associated with 3 arterial function measures whereas waist circumference and waist-to-hip ratio related to only 1 of the respective variables. Also, among markers of cumulative inflammation, disease duration was associated with 6 arterial function measures whereas deformed joint count related to only 1 of the respective measures. Recorded characteristics that were not associated with arterial function are given in Supplementary Table 1, available from the authors on request.

Table 2. Arterial function among patients with rheumatoid arthritis from different population groups. Results are expressed as mean (SD) and median (interquartile range) for normally and non-normally distributed characteristics, respectively.

Measurement	White, n = 118	Asian, n = 32	Black, n = 22	Mixed, n = 5	p
Central systolic BP, mmHg	127 (16)	123 (15)	125 (15)	131 (17)	0.5
Central pulse pressure, mmHg	44 (14)	37 (11)	39 (13)	46 (11)	0.02
Pulse wave velocity, m/s	7.3 (5.9–10.0)	7.2 (6.3–8.3)	7.3 (6.2–8.5)	7.5 (3.3–15.0)	0.7
Augmentation index, %	30 (11)	32 (12)	37 (11)	31 (7)	0.08
Forward wave pressure, mmHg	29 (8)	25 (8)	24 (9)	32 (13)	0.01
Reflected wave pressure, mmHg	22 (8)	17 (5)	19 (7)	22 (4)	0.02
Reflection magnitude, %	75 (24)	70 (19)	81 (26)	74 (16)	0.4
Pulse pressure amplification	1.22 (1.15–1.32)	1.24 (1.17–1.32)	1.22 (1.16–1.28)	1.21 (1.12–1.42)	0.9
Peripheral pulse pressure, mmHg	48 (13)	42 (10)	44 (17)	49 (19)	0.07

Intergroup comparisons were made by the ANOVA test with logarithmic transformation of pulse wave velocity and pulse pressure amplification. Significant disparities are shown in bold. BP: blood pressure.

Table 3. Bivariate relations among arterial function measurements in 177 patients with rheumatoid arthritis. Results are expressed as Pearson correlation coefficients (p).

	Pulse Wave Velocity	Augmentation Index	Reflected Wave Pressure	Reflection Magnitude	Central Systolic BP	Central Pulse Pressure	Peripheral Pulse Pressure	Pulse Pressure Amplification	Forward Wave Pressure
Pulse wave velocity	1								
Augmentation index	0.10 (0.2)	1							
Reflected wave pressure	0.27 (< 0.01)	0.54 (< 0.01)	1						
Reflection magnitude	0.07 (0.4)	0.78 (< 0.01)	0.62 (< 0.01)	1					
Central systolic BP	0.39 (< 0.01)	0.35 (< 0.01)	0.76 (< 0.01)	0.30 (< 0.01)	1				
Central pulse pressure	0.28 (< 0.01)	0.42 (< 0.01)	0.96 (< 0.01)	0.42 (< 0.01)	0.80 (< 0.01)	1			
Peripheral pulse pressure	0.29 (< 0.01)	0.21 (0.01)	0.66 (< 0.01)	0.25 (< 0.01)	0.64 (< 0.01)	0.68 (< 0.01)	1		
Pulse pressure amplification	–0.15 (0.06)	–0.76 (< 0.01)	–0.59 (< 0.01)	–0.70 (< 0.01)	–0.37 (< 0.01)	–0.48 (< 0.01)	–0.18 (0.02)	1	
Forward wave pressure	0.28 (< 0.01)	–0.04 (0.6)	–0.16 (0.03)	–0.16 (0.03)	0.66 (< 0.01)	0.80 (< 0.01)	0.57 (< 0.01)	–0.06 (0.4)	1

Significant associations are shown in bold. ¹Logarithmically transformed. BP: blood pressure.

Table 4. Associations of baseline characteristics with arterial function in 177 patients with rheumatoid arthritis.

Characteristic	Arterial Stiffness				Wave Reflection				Pressure Pulsatility								Forward wave pressure	
	Pulse wave velocity		Augmentation index		Reflected wave pressure		Reflection magnitude		Central systolic BP		Central pulse pressure		Peripheral pulse pressure ¹		Pulse pressure amplification			
	Partial r	p	Partial r	p	Partial r	p	Partial r	p	Partial r	p	Partial r	p	Partial r	p	Partial r	p		
Age	0.37	<0.01	0.03	0.7	0.30	<0.01	0.10	0.2	0.20	0.01	0.31	<0.01	0.38	<0.01	-0.13	0.1	0.22	0.01
Female	-0.09	0.3	0.22	0.01	0.14	0.08	0.19	0.01	0.01	0.9	0.09	0.2	0.07	0.4	-0.17	0.03	-0.05	0.5
Heart rate	0.05	0.6	-0.42	<0.01	-0.54	<0.01	-0.56	<0.01	-0.24	<0.01	-0.41	<0.01	0.04	0.6	0.70	<0.01	0.08	0.3
Body height	0.02	0.8	-0.19	0.02	-0.17	0.03	-0.17	0.03	-0.06	0.5	-0.14	0.08	-0.04	0.6	0.19	0.01	-0.05	0.5
SBP	0.15	0.06	0.20	0.01	0.64	<0.01	0.23	<0.01	0.66	<0.01	0.63	<0.01	—	—	-0.16	0.04	0.47	<0.01
DBP	0.06	0.5	0.03	0.7	-0.36	<0.01	-0.06	0.5	0.07	0.4	-0.37	<0.01	—	—	-0.05	0.5	-0.28	<0.01
Exercise	-0.01	0.9	-0.11	0.2	-0.10	0.2	-0.06	0.5	-0.15	0.05	-0.11	0.2	-0.12	0.1	0.10	0.2	-0.07	0.4
Alcohol	0.06	0.5	0.09	0.2	-0.01	0.9	0.11	0.1	-0.04	0.7	-0.04	0.6	-0.16	0.02	-0.13	0.1	-0.10	0.2
BMI ²	-0.11	0.2	-0.22	<0.01	-0.19	0.02	-0.13	0.09	-0.14	0.08	-0.13	0.1	0.01	0.9	0.23	<0.01	-0.02	0.8
Waist	-0.06	0.5	-0.15	0.07	-0.12	0.1	-0.09	0.3	-0.03	0.7	-0.07	0.4	0.02	0.8	0.19	0.02	-0.02	0.8
WHR	0.05	0.6	-0.06	0.4	-0.06	0.5	-0.03	0.7	0.06	0.5	-0.06	0.5	0.03	0.7	0.18	0.02	-0.06	0.5
Statin	0.06	0.5	-0.04	0.6	0.04	0.6	-0.08	0.3	0.21	0.01	0.10	0.2	0.05	0.5	0.09	0.2	0.10	0.2
Insulin treatment	-0.07	0.4	0.10	0.2	0.00	1.0	0.10	0.2	-0.16	0.04	-0.01	0.9	0.16	0.04	-0.12	0.1	-0.06	0.4
RA duration ³	0.18	0.03	0.09	0.3	0.29	<0.01	0.14	0.07	0.20	0.01	0.28	<0.01	0.21	0.01	-0.12	0.1	0.17	0.03
CRP	-0.13	0.1	-0.07	0.4	-0.10	0.2	0.08	0.3	-0.12	0.1	-0.16	0.04	0.17	0.03	0.08	0.3	-0.16	0.05
Deformed joint	0.01	0.9	0.07	0.4	0.14	0.08	0.06	0.5	0.19	0.01	0.15	0.06	-0.00	1.0	-0.02	0.8	0.13	0.1
RF-positive	0.02	0.9	0.09	0.3	0.17	0.03	0.10	0.22	0.06	0.4	0.14	0.07	0.07	0.4	-0.17	0.03	0.08	0.3
WCC	0.00	1.0	0.10	0.1	0.15	0.07	0.17	0.03	0.09	0.3	0.11	0.2	0.10	0.2	-0.12	0.1	-0.01	0.9
Methotrexate	0.16	0.05	-0.05	0.6	0.03	0.7	-0.06	0.5	0.04	0.6	0.08	0.3	0.50	0.6	-0.01	0.9	0.11	0.2
Chloroquine	0.02	0.8	0.01	0.9	-0.03	0.7	0.03	0.7	-0.08	0.3	-0.04	0.6	-0.17	0.03	-0.02	0.8	-0.04	0.6
Tetracycline	0.07	0.4	-0.02	0.8	0.03	0.7	-0.07	0.4	0.05	0.5	0.06	0.5	-0.18	0.02	0.01	0.9	0.10	0.2
TNF-α inhibitor	-0.19	0.02	-0.14	0.08	-0.06	0.5	-0.09	0.3	-0.04	0.6	-0.01	0.9	0.05	0.5	0.16	0.04	0.04	0.6
CKD-EPI ⁴	-0.28	<0.01	0.16	0.07	-0.05	0.6	0.07	0.4	-0.03	0.7	-0.09	0.3	-0.25	<0.01	-0.10	0.2	-0.13	0.1
Total cholesterol	0.02	0.8	0.20	0.01	0.03	0.7	0.21	0.01	0.01	0.9	-0.04	0.6	0.07	0.4	-0.20	0.01	-0.13	0.09
HDL cholesterol	0.09	0.3	0.20	0.01	0.14	0.08	0.22	<0.01	-0.07	0.4	0.05	0.6	0.04	0.6	-0.22	<0.01	-0.08	0.3
HOMA-IR	-0.03	0.8	-0.20	0.02	-0.07	0.4	-0.07	0.4	-0.03	0.7	-0.02	0.8	0.11	0.2	0.09	0.3	-0.00	1.0

Associations were assessed in age, sex, race, body height, heart rate, and systolic and diastolic BP adjusted linear regression models with exceptions as stated below. Non-normally distributed variables were log transformed prior to being entered in the models. Significant associations are shown in bold. ¹Systolic and diastolic BP were not included in the models because these characteristics form part of brachial BP measurements. ²Body height was not entered into the models because this characteristic is included in BMI calculation. ³Age at time of the study was replaced by age at disease onset, to avoid duplication. ⁴Age, sex, and race were not entered into the models because these characteristics are included in CKD-EPI calculation. SBP: systolic blood pressure; DBP: diastolic blood pressure; BMI: body mass index; RA: rheumatoid arthritis; WHR: waist-hip ratio; CRP: C-reactive protein; RF: rheumatoid factor; WCC: white cell (leukocyte) count; TNF: tumor necrosis factor; CKD-EPI: Chronic Kidney Disease Epidemiology Collaboration; HDL: high-density lipoprotein; HOMA-IR: homeostatic model assessment for insulin resistance.

Independent relationships of patient characteristics with arterial function. In view of the large number of associations between recorded characteristics and arterial function measures in the previous analysis, patient characteristic–arterial function relationships were further evaluated in stepwise backward regression models. These results are given in Table 5. Except for waist circumference, waist-to-hip ratio, and deformed joint count, each patient characteristic shown in Table 3 was entered consistently into the models; race was additionally forced into the models. The CKD-EPI was rejected by the analysis owing to co-linearity with age.

Besides HDL cholesterol concentrations and chloroquine, all analyzed characteristics were independently associated with at least 1 and up to 8 of the 9 evaluated arterial function measures. Age and brachial systolic or mean BP related to large, and body height and diastolic BP to low measures of arterial function, as reported in non-RA populations. Race

was also directly associated with augmentation index and inversely to peripheral pulse pressure. In this regard, the mean (SD) augmentation index values were lower in whites [130 (11)] compared to patients from other population groups [131 (7) to 137 (11); Table 2] whereas peripheral BP was larger in white [48 (13)] than in Asian [42 (10)] and black [44 (17)] RA participants. Exercise, alcohol consumption, and insulin therapy were associated with favorable, and total cholesterol concentrations with unfavorable arterial function measures. Statin therapy was paradoxically associated with increased central systolic BP (partial $r = 0.28$, $p < 0.01$). The mean (SD) Framingham score was 4.1 (6.4) and 2.1 (4.5) in patients taking statins compared to patients not taking them ($p < 0.01$), respectively. BMI (partial $r = -0.19$, $p = 0.02$) and HOMA-IR (partial $r = -0.20$, $p = 0.01$) were each paradoxically related to low augmentation index values. BMI was further paradoxically associated with a large pulse pressure

Table 5. Independent relationships of baseline characteristics with arterial function in 177 patients with rheumatoid arthritis.

Characteristic	Arterial Stiffness				Wave Reflection				Pressure Pulsatility								Forward wave pressure	
	Pulse wave velocity		Augmentation index		Reflected wave pressure		Reflection magnitude		Central systolic BP		Central pulse pressure		Peripheral pulse pressure ¹		Pulse pressure amplification			
	Partial r	p	Partial r	p	Partial r	p	Partial r	p	Partial r	p	Partial r	p	Partial r	p	Partial r	p	Partial r	p
Age	0.27	<0.01	—	—	0.29	<0.01	—	—	—	—	0.33	<0.01	0.24	<0.01	—	—	0.31	<0.01
Female	−0.22	0.01	0.16	0.05	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Race ²	—	—	0.21	0.01	—	—	—	—	—	—	—	—	−0.28	<0.01	—	—	—	—
Body height	—	—	−0.27	<0.01	−0.39	<0.01	−0.43	<0.01	—	—	−0.32	<0.01	—	—	0.42	<0.01	—	—
Heart rate	—	—	−0.43	<0.01	−0.56	<0.01	−0.62	<0.01	−0.27	<0.01	−0.41	<0.01	—	—	0.74	<0.01	0.16	0.05
SBP	0.27	<0.01	0.32	<0.01	0.66	<0.01	0.26	<0.01	0.79	<0.01	0.67	<0.01	—	—	−0.25	<0.01	0.51	<0.01
DBP	—	—	—	—	−0.34	<0.01	—	—	—	—	−0.37	<0.01	—	—	—	—	−0.31	<0.01
Mean BP	—	—	—	—	—	—	—	—	—	—	—	—	0.46	<0.01	—	—	—	—
BMI ³	—	—	−0.19	0.02	—	—	—	—	—	—	—	—	−0.29	<0.01	0.20	0.01	—	—
Exercise	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.19	0.02	—	—
Alcohol	—	—	—	—	—	—	—	—	—	—	—	—	−0.27	<0.01	—	—	—	—
Statin	—	—	—	—	—	—	—	—	0.28	<0.01	—	—	—	—	—	—	—	—
Insulin Rx	—	—	—	—	−0.24	<0.01	—	—	−0.30	<0.01	−0.29	<0.01	—	—	−0.19	0.02	−0.30	<0.01
RA duration ⁴	—	—	—	—	—	—	0.17	0.04	—	—	—	—	—	—	—	—	—	—
CRP	−0.17	0.05	—	—	—	—	—	—	—	—	−0.24	<0.01	—	—	—	—	−0.20	0.01
RF-positive	—	—	—	—	0.20	0.01	—	—	—	—	0.18	0.03	—	—	−0.19	0.02	—	—
WCC	—	—	—	—	0.16	0.05	0.19	0.02	—	—	0.16	0.05	—	—	—	—	—	—
Methotrexate	0.19	0.03	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Chloroquine	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Tetracycline	—	—	—	—	—	—	—	—	—	—	—	—	−0.20	0.02	—	—	—	—
TNF-α	−0.25	<0.01	—	—	—	—	—	—	—	—	—	—	—	—	0.23	<0.01	—	—
Total chol.	—	—	0.24	<0.01	—	—	0.26	<0.01	—	—	—	—	—	—	−0.18	0.03	—	—
HDL chol.	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
HOMA-IR	—	—	−0.20	0.01	—	—	—	—	—	—	—	—	−0.21	<0.01	—	—	—	—
Model R ²	0.37		0.48		0.71		0.48		0.68		0.68		0.48		0.63		0.46	

Significant associations are shown in bold. Relationships were assessed in stepwise backward regression models; all patient characteristics (other than waist circumference, waist-to-hip ratio, and no. deformed joints) were consistently entered as independent variables with exceptions as stated below, and race was additionally forced into the models. The Chronic Kidney Disease Epidemiology Collaboration was rejected in the analysis because of co-linearity with age. ¹Systolic and diastolic blood pressure were replaced by mean arterial pressure in the model. ²White = 1; Asian = 2; black = 3; mixed = 4. ³Height was not entered into the model. ⁴Age at time of the study was replaced by age at disease onset in the model. SBP: systolic blood pressure; DBP: diastolic blood pressure; BP: blood pressure; BMI: body mass index; Rx: treatment; RA: rheumatoid arthritis; CRP: C-reactive protein; RF: rheumatoid factor; WCC: white cell (leukocyte) count; TNF: tumor necrosis factor; chol: cholesterol; HDL: high-density lipoprotein; HOMA-IR: homeostatic model assessment for insulin resistance.

amplification (partial $r = 0.20$, $p = 0.01$) and HOMA-IR with peripheral pulse pressure (partial $r = -0.21$, $p < 0.01$).

Among RA characteristics, disease duration was associated with reflection magnitude (partial $r = 0.17$, $p = 0.04$) and RF status was related to central pulse pressure (partial $r = 0.18$, $p = 0.03$), reflected wave pressure (partial $p = 0.20$, $p = 0.01$), and pulse pressure amplification (partial $r = -0.19$, $p = 0.02$). Leukocyte count was also directly related to central pulse pressure (partial $r = 0.16$, $p = 0.05$), reflected wave pressure (partial $r = 0.16$, $p = 0.05$), and reflection magnitude (partial $r = 0.19$, $p = 0.02$). In this regard, the leukocyte count was associated with the logarithmically transformed CDAI (Pearson $r = 0.21$, $p < 0.01$), DAS28 (Pearson $r = 0.19$, $p = 0.01$), and logarithmically transformed CRP concentrations (Pearson $r = 0.34$, $p < 0.01$). Neither RA duration nor RF status and leukocyte count were related to pulse wave velocity and augmentation index.

CRP concentrations were paradoxically related to low central pulse pressure (partial $r = -0.24$, $p < 0.01$), pulse wave velocity (partial $r = -0.17$, $p = 0.05$), and forward wave pressure (partial $r = -0.20$, $p = 0.01$) values.

Regarding RA treatment, TNF- α inhibition was associated with reduced pulse wave velocity (partial $r = -0.25$, $p < 0.01$) and increased pulse pressure amplification (partial $r = 0.23$, $p < 0.01$) whereas tetracycline use (partial $r = -0.20$, $p = 0.02$) was related to low peripheral pulse pressure. MTX use was paradoxically associated with large pulse wave velocity (partial $r = 0.19$, $p = 0.03$). The joint deformity count tended to be larger in patients using MTX [median (IQR) = 1 (0–11)] compared to those who did not [median (IQR) = 0 (0–3); $p = 0.08$]. Therefore, MTX use may have represented a disease severity marker in the present context.

Overall, the recorded patient characteristics explained 37% (pulse wave velocity) to 71% (reflected wave pressure) of the variability in arterial stiffness.

DISCUSSION

Our present study explored the potential effect of a wide range of traditional cardiovascular risk factors and disease characteristics on different aspects of arterial function in patients with RA. The data analysis produced several novel findings. In previous RA studies, pulse wave velocity and augmentation index were the most frequently investigated central hemodynamic measures²¹. In our investigation, disease duration that represents cumulative inflammation and RF positivity that characterizes severe RA²⁵ were associated with central pulse pressure and wave reflection markers independent of brachial BP and other potential confounders, but unrelated to pulse wave velocity or augmentation index. RF status was further associated with reduced pulse pressure amplification, which also predicts incident cardiovascular events in non-RA populations^{3,4,16}. Additionally, the leukocyte count that represented disease activity in this RA population was similarly associated with central pulse pressure and wave reflection, not with pulse wave velocity or augmentation index. Leukocyte count as an inflammatory marker was previously reported to represent an indicator of arterial stiffness in non-RA persons²⁶ and atherosclerosis in patients with RA²⁷.

Associations of cumulative inflammation, and disease activity and severity as assessed by area under the curve erythrocyte sedimentation rate (ESR), CDAI, or DAS28 and Health Assessment Questionnaire–Disability Index (HAQ-DI), respectively, with arterial stiffness were reported in some^{28,29,30} but not all³¹ previous RA studies. In our present study, in contrast to leukocyte counts, no relationships were identified between CDAI or DAS28 and HAQ-DI and any of the 9 evaluated arterial function measures. However, the majority of patients in the present investigation experienced either low disease activity or remission, which could have accounted for our findings.

We found that RA treatment related to arterial stiffness. TNF- α inhibition was associated with reduced pulse wave velocity, in keeping with findings in most previous RA studies³². TNF- α inhibition was also associated with increased BP amplification in our present study. Interestingly, Vassilopoulos and colleagues³³ reported a reduction in pulse wave velocity with adalimumab that occurred irrespective of treatment response and was unrelated to decreases in DAS28 among patients with RA. We further report for the first time, to our knowledge, that tetracycline use is associated with a reduced peripheral pulse pressure in RA. In this regard, matrix metalloproteinases (MMP) play a pivotal role in vascular changes induced by HTN and arterial stiffness³⁴. Doxycycline, the tetracycline used in our patients, comprises a potent MMP inhibitor and reduces functional and structural alterations in HTN as well as arterial stiffness³⁴. The potential role of doxycycline in cardiovascular risk prevention in RA merits further longitudinal investigation.

BMI and insulin resistance were associated with reduced

augmentation index and peripheral pulse pressure. BMI additionally related to increased pulse pressure amplification. An inverse relationship of adiposity and insulin resistance with arterial function markers has been increasingly documented in non-RA subjects^{35,36,37,38}. Indeed, excess adiposity is associated with reduced wave reflection in indigenous Australians³⁵ as well as Korean subjects³⁶ with metabolic syndrome. In indigenous Australians, fasting insulin concentrations were also related to decreased wave reflection³⁵. Circulating concentrations of free fatty acids that are major initiators of insulin resistance are associated with a reduced augmentation index and central systolic BP and increased pulse pressure amplification³⁷. The Toon Health study reported an inverse relationship between insulin resistance and central BP³⁸. Upon applying separation analysis in diabetic patients, Chirinos and colleagues³⁹ recently reported reduced wave reflection that may account for increased penetration of pulsatile energy to distal vascular beds, and thereby enhance microvascular disease. Compensatory hyperinsulinemia in response to insulin resistance may induce vasodilation, leading to reduced central pressures and wave reflection³⁸. In this regard, insulin treatment was also associated with reduced central pressures and wave reflection in our present study. Although insulin administration lowers arterial stiffness in healthy as well as diabetic persons⁴⁰, the latter finding should be interpreted with caution because only 3 patients with RA used insulin in this investigation.

A third of our patients exercised regularly and/or consumed low or moderate alcohol quantities (data not shown). A metaanalysis documented that aerobic exercise reduces pulse wave velocity and the augmentation index in non-RA persons⁴¹. Individualized exercise was documented to reduce cardiovascular risk in RA⁴². Light and moderate alcohol consumption is associated with pulse wave velocity in non-RA subjects⁴³. In this study, exercise was related to pulse pressure amplification and alcohol consumption to reduced peripheral pulse pressure.

Hypercholesterolemia increases arterial stiffness and central but not peripheral BP⁴⁴. Total and low-density lipoprotein cholesterol as well as triglyceride concentrations were both related to central BP and wave reflection markers in previous non-RA studies⁴⁵. In the present investigation, total cholesterol concentrations were independently associated with increased augmentation index and reflection magnitude, and reduced pulse pressure amplification.

Arterial stiffness and wave reflections are the most important determinants of systolic and pulse pressure^{10,46}. In concert, these hemodynamic changes increase cardiac workload and affect coronary, cerebral, and renal blood flow, and are associated with atherosclerosis^{10,46}. Our results suggest that lifestyle intervention, adequate treatment of traditional cardiovascular risk factors, and disease control may reduce cardiovascular risk by improving arterial function in RA. Notably in this regard, we found more

consistent relationships of cardiovascular risk factors and RA characteristics with wave reflection and pressure pulsatility than with arterial stiffness.

We evaluated a comprehensive panel of arterial function measures and consistently adjusted for recognized and newly identified potential confounders. However, we assessed a large number of associations and used backward regression models on data obtained in a study with a cross-sectional design, which precludes drawing conclusions on the direction of causality. These limitations may have contributed to our finding of an inverse relationship of CRP concentrations with pulse wave velocity and central pulse pressure, and a direct association of statin therapy with central systolic BP. Indeed, CRP concentrations are reportedly associated with increased pulse wave velocity in RA²¹ and several studies have shown a beneficial effect of statin therapy on arterial stiffness in non-RA persons⁴⁷, as well as in those with RA⁴⁸. We did not include a healthy control group, and future interventional studies are clearly indicated. Cumulative ESR values and CRP concentrations²⁸ represent better markers of inflammation accrued over time than disease duration, but were not recorded in the present study.

Traditional cardiovascular risk factors and disease characteristics are consistently related to vascular hemodynamic alterations in RA. The relative effect of arterial stiffness, wave reflection, and pressure pulsatility in the enhanced CVD risk among patients with RA needs further investigation.

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Online Supplementary Data

Supplementary Table 1. *Baseline characteristics that were not associated with central pressures and arterial stiffness measures in 177 rheumatoid arthritis patients.*

	Central systolic pressure		Central pulse pressure		Pulse wave velocity		Augmentation index		Forward wave pressure		Reflected wave pressure		Reflection magnitude		Pulse pressure amplification		Peripheral pulse pressure ¹	
Characteristic	partial r	p	partial r	P	partial r	p	partial r	p	partial r	p	partial r	p	partial r	p	partial r	p	partial r	p
Race ²	0.03	0.7	-0.06	0.5	0.02	0.8	0.14	0.08	-0.09	0.3	-0.06	0.5	0.07	0.4	-0.06	0.4	-0.08	0.3
Smoking	0.10	0.2	-0.05	0.6	0.01	0.9	0.14	0.08	-0.10	0.2	0.01	0.9	0.10	0.2	-0.15	0.06	-0.01	0.9
Ezetimibe	0.07	0.4	0.07	0.4	0.05	0.6	-0.03	0.7	0.01	0.9	0.10	0.2	0.04	0.6	0.11	0.2	-0.06	0.4
OGLA	-0.13	0.1	-0.11	0.2	-0.08	0.3	0.03	0.7	-0.13	0.1	-0.08	0.3	0.03	0.7	-0.06	0.5	0.02	0.8
CDAI	0.06	0.5	-0.02	0.8	-0.06	0.5	0.05	0.5	0.02	0.8	-0.04	0.6	-0.02	0.9	-0.08	0.3	0.06	0.5
DAS28	0.02	0.8	-0.06	0.5	-0.10	0.2	-0.04	0.6	0.00	1.0	-0.08	0.3	-0.05	0.5	-0.05	0.5	0.06	0.4
ESR	-0.02	0.8	-0.01	0.9	-0.15	0.09	-0.10	0.2	0.04	0.6	-0.02	0.8	-0.04	0.6	-0.03	0.7	0.08	0.3
ACPA	0.06	0.4	0.10	0.2	-0.01	0.9	0.02	0.8	0.09	0.3	0.06	0.4	-0.02	0.8	-0.02	0.8	0.07	0.4
EA manifestations	0.10	0.2	0.07	0.4	0.05	0.5	0.02	0.8	0.06	0.4	0.10	0.2	0.03	0.7	0.00	1.0	0.02	0.8
HAQ-DI	0.05	0.6	-0.03	0.7	-0.07	0.4	-0.05	0.6	0.07	0.4	-0.09	0.2	-0.10	0.2	0.07	0.4	-0.07	0.4
Leflunomide	0.03	0.7	0.04	0.6	0.04	0.6	0.02	0.8	0.07	0.4	0.02	0.8	-0.10	0.2	0.05	0.6	-0.04	0.6
Sulphasalazine	0.07	0.4	0.01	0.9	0.05	0.5	-0.07	0.4	0.04	0.7	-0.01	0.9	-0.06	0.5	0.04	0.6	-0.09	0.2
Azathioprine	0.03	0.7	0.06	0.5	-0.03	0.7	0.08	0.3	0.01	0.9	0.07	0.4	0.09	0.3	-0.09	0.3	-0.13	0.1
Abatacept	-0.03	0.7	-0.04	0.6	0.11	0.2	-0.07	0.4	-0.04	0.6	-0.04	0.6	-0.04	0.7	0.09	0.2	-0.09	0.3
NSAIDs	-0.08	0.3	0.02	0.8	0.03	0.7	0.00	1.0	0.07	0.4	0.03	0.7	-0.04	0.6	0.05	0.6	0.10	0.2
Prednisone	0.06	0.4	0.06	0.5	0.03	0.8	0.07	0.4	-0.01	0.9	0.08	0.3	0.08	0.3	0.00	1.0	0.06	0.5
Total C/HDL C	0.11	0.2	-0.08	0.3	-0.06	0.5	-0.03	0.7	-0.04	0.6	-0.10	0.2	-0.04	0.6	0.09	0.3	0.06	0.5
Total C/HDL C >4	0.12	0.1	-0.03	0.7	-0.08	0.3	0.03	0.3	-0.03	0.8	-0.04	0.6	-0.04	0.6	-0.01	0.9	0.13	0.1
LDL cholesterol	0.05	0.6	-0.07	0.4	-0.02	0.8	0.10	0.2	-0.09	0.3	-0.05	0.5	0.10	0.2	-0.15	0.07	0.09	0.2
Triglycerides	-0.03	0.7	-0.04	0.6	-0.04	0.5	0.00	1.0	-0.07	0.4	-0.02	0.8	0.05	0.5	0.10	0.2	-0.00	1.0
Diabetes	0.09	0.3	0.12	0.1	-0.05	0.5	0.09	0.2	0.05	0.6	0.1	0.2	0.08	0.3	-0.1	0.2	0.12	0.1
Glucose	-0.10	0.2	-0.05	0.5	-0.04	0.6	-0.07	0.4	-0.09	0.3	-0.01	0.9	0.08	0.3	0.06	0.4	0.04	0.6

Associations were assessed in age, gender, race, body height, heart rate and systolic and diastolic blood pressure adjusted linear regression models with one exception as stated below (¹).

Non-normally distributed variables were log transformed prior to entering them in the models.

OGLA: oral glucose lowering agents; CDAI: clinical disease activity score; DAS 28: disease activity score in 28 joints; ESR: erythrocyte sedimentation rate; ACPA: anti-citrullinated peptide antibody; EA: extra-articular; HAQ-DI: Stanford Health Assessment Questionnaire disability index; NSAIDs: non-steroidal anti-inflammatory drugs; C: cholesterol; HDL: high density lipoprotein; LDL: low-density lipoprotein.

¹Systolic and diastolic blood pressure were not included in the models.

²White=1; Asian=2; Black=3; Mixed=4.

Paper 1 – Cardiovascular risk factors and disease characteristics are consistently associated with arterial function in rheumatoid arthritis

Paper 1 showed that RA disease characteristics and cardiovascular risk factors are consistently associated with impaired arterial function including arterial stiffness and increased wave reflection and pressure pulsatility. Impaired arterial function may therefore represent a link between RA and the reported increased risk of atherosclerotic CVD. Can impaired arterial function impact on atherosclerosis in patients with RA and, if this is the case, which arterial properties are most important in the present context?

Arterial wave reflection and subclinical atherosclerosis in rheumatoid arthritis

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Running title: Wave reflection and atherosclerosis in RA

Abstract

Objective. Atherosclerotic cardiovascular disease risk is increased in rheumatoid arthritis (RA). Wave reflection occurs at arterial branching points, which are particularly prone to atherosclerosis. We explored the relationship of wave reflection with atherosclerosis in RA.

Methods. One hundred and sixty three RA patients (110 white, 31 Asian, 17 black and 5 of mixed ancestry) without cardiovascular disease participated. Arterial stiffness, wave reflection, pressure pulsatility, plaque in the extracranial carotid artery tree and the mean of the left and right common carotid arteries intima-thickness were determined. Associations were identified in multivariable regression models.

Results. One SD increase in reflected wave pressure (OR (95% CI) = 2.54 (1.41-4.44), $p = 0.001$), reflection magnitude (OR (95% CI) = 1.84 (1.17-2.89), $p = 0.008$), central pulse pressure (OR (95% CI) = 1.89 (1.12-3.22), $p = 0.02$) and peripheral pulse pressure (OR (95% CI) = 2.09 (1.23-3.57), $p = 0.007$) were associated with plaque. The association of wave reflection with plaque was independent of arterial stiffness and pressure pulsatility, and was present in both hypertensive and normotensive RA patients. In receiver operator characteristic curve analysis, the optimal cutoff value for reflected wave pressure in predicting plaque presence was 25 mmHg with a sensitivity, specificity, positive predictive value and negative predictive value of 45.2%, 89.3%, 78.6% and 66.2%, respectively; a reflected wave pressure of >25 mmHg was associated with plaque in univariate and adjusted analysis ($p < 0.0001$ for both). Arterial function was not independently related to carotid intima-media thickness.

Conclusion. Consideration and therapeutic targeting of wave reflection may improve cardiovascular disease prevention in RA.

Key words: wave reflection, arterial stiffness, pressure pulsatility, atherosclerosis, rheumatoid arthritis

Introduction

Aging and exposure to cardiovascular risk factors is associated with impaired arterial function (1-14). This results in hemodynamic changes that increase left ventricular afterload and reduce coronary blood flow (1-14). Impaired arterial function predicts end organ damage, heart failure and cardiovascular mortality (1-14).

The state-of-the-art estimation of arterial stiffness is by measurement of pulse wave velocity (5). However, besides age and blood pressure, there is little or no association between cardiovascular risk factors and pulse wave velocity (13). Whether arterial stiffness is related to atherosclerosis in non-RA subjects remains uncertain as studies addressing this association have produced conflicting results (13). In this regard, increased arterial wave reflection and pressure pulsatility represent other arterial properties that predict cardiovascular event rates (1-5, 7-14). Wave reflection occurs at arterial branching points and is further influenced by arterial wall content (5,7-9).

The increased risk of atherosclerotic cardiovascular disease in rheumatoid arthritis (RA) is well documented (15). Patients with RA categorized as having moderate cardiovascular risk when risk charts are used often have carotid plaques (16). This finding unveils the high subclinical atherosclerotic burden observed in these patients. A recent meta-analysis reported enhanced pulse wave velocity and augmentation index in RA (17). Avalos and colleagues (18) found that the augmentation index was related to coronary artery calcification in RA but this association was attenuated ($p=0.09$) upon adjustment for potential confounders. We recently reported that pulse wave velocity and augmentation index are not independently associated with carotid atherosclerosis in Spanish patients with RA (19). Arterial branching points are the sites that are particularly prone to atherosclerosis due to a departure from the axially aligned unidirectional flow and reduced wall shear stress (20,21). In this study, we hypothesised that wave reflection as assessed in separation analysis (5,7-10), is associated with atherosclerosis in RA. Additionally, we aimed to validate our previously reported findings on the lack of association of pulse wave velocity and augmentation index with atherosclerosis in patients with RA (19).

Patients and methods

Patients

We enrolled 163 consecutive patients (110 white, 31 Asian, 17 black and 5 of mixed ancestry) that fulfilled the 2010 ACR/European League Against Rheumatism (EULAR) criteria for RA (22) and were free of established cardiovascular disease at the Milpark Hospital, Johannesburg, South Africa. This cross-sectional study obtained approval from the University of Witwatersrand Human (Medical) Research Ethics Committee (approval number: M06-07-33; protocol number: M120562) in Johannesburg, South Africa. Each participant gave written, informed consent.

Baseline characteristics

Standardized methods were used to record baseline characteristics (23). Briefly, demographic features, lifestyle factors, anthropometric measures, metabolic risk factors, RA characteristics and cardiovascular and antirheumatic agent use were evaluated. Disease activity was estimated by the Clinical Disease Activity Index (CDAI) and Disease Activity Score in 28 joints (DAS28), and physical impairment by the Stanford Health Assessment Questionnaire disability index. C-reactive protein was measured by immunoturbidimetry and insulin using a chemiluminescent microparticle immunoassay (Abbott Laboratories, Abbott Park, IL 60064 USA).

Hypertension was diagnosed when the average systolic blood pressure of ≥ 140 and/or diastolic blood pressure of ≥ 90 mmHg and/or anti-hypertensive medication was employed. Dyslipidemia was identified when the atherogenic index, i.e., the cholesterol/high density lipoprotein (HDL) ratio was > 4 (15) and/or lipid lowering agents were used. Diabetes was diagnosed in patients that employed glucose lowering agents or had a fasting plasma glucose of ≥ 7 mmol/l. The glomerular filtration rate was estimated by the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation (24) and the homeostatic model assessment of insulin resistance (25) was calculated.

Arterial function measurements

Central hemodynamic parameters were evaluated using radial applanation tonometry and SphygmoCor software (26). Pulse wave velocity (27), aortic blood pressure and reflected and

forward wave pressures were determined using a high-fidelity SPC-301 micromanometer (Millar instrument, Inc., Houston, Texas), interfaced with a computer utilizing SphygmoCor software, version 9.0 (AtCor Medical Pty. Ltd., West Ryde, New South Wales, Australia). After resting for 15 minutes in the supine position, arterial waveforms at the radial (dominant arm), carotid and femoral artery pulses were recorded for a time period of 10 consecutive waveforms (heart beats). The pulse wave was calibrated by manual measurement (auscultation) of brachial BP taken immediately before the recordings. Using a validated generalized transfer function incorporated in the SphygmoCor software, the peripheral pressure waveform was converted into a central aortic waveform. When systolic or diastolic variability of consecutive waveforms exceeded 5% or the amplitude of the pulse wave signal was less than 80 mV, the recorded results were discarded. Aortic pulse wave velocity was determined from sequential waveform measurements at carotid and femoral sites as previously described. The time delay in pulse waves between the carotid and femoral sites was determined using an ECG-derived R wave as a fiducial point. Pulse transit time was taken as the average of 10 consecutive beats. The distance which the pulse wave travels was determined as the difference between the distance from the femoral sampling site to the suprasternal notch and the distance from the carotid sampling site to the suprasternal notch.

The carotid-femoral pulse wave velocity was calculated as the distance between the carotid and femoral pulses in meters divided by transit time in seconds. Central systolic blood pressure was derived from the aortic waveform and central aortic pulse pressure was calculated from the difference between central systolic blood pressure and central diastolic blood pressure. The magnitude of the forward and reflected wave components of the aortic pressure waveform was determined by the SphygmoCor software that separates the aortic waveform by employing a modified triangular waveform. Augmentation index was defined as the augmented pressure (difference between second and first systolic shoulder) expressed as a percentage of central pulse pressure. Reflection magnitude was calculated as (reflected wave amplitude/forward wave amplitude) x 100 and pulse pressure amplification as the radial pulse pressure divided by the aortic pulse pressure. All measurements were made by a single experienced technician who was unaware of the cardiovascular risk factor profiles of the patients. Brachial pressures were obtained

in all patients and technically sound wave reflection, and pulse wave velocity measurements in 157 and 150 participants, respectively. The intra-observer variability of tonometry measurements is low in our setting (26).

Carotid artery ultrasound

Carotid artery ultrasound was performed using high resolution B-mode ultrasound (LOGIC E9, GE Healthcare, USA) with software that provides semi-automated border detection and markedly reproducible data. Images of at least 1cm length of the distal common carotid arteries were obtained using a linear array 5-13 MHz probe. The optimal angle of incidence was used, defined as the longitudinal angle of approach where both branches of the internal and external carotid artery are visualized simultaneously. The carotid intima-media thickness (c-IMT) was defined as the mean of the left and right common carotid arteries thickness. Plaque in the extracranial carotid tree was defined according to the Mannheim consensus criteria (28). A single qualified technician who was blinded to the cardiovascular risk profiles of the patients, performed all carotid ultrasound measurements. The intra-observer variability of ultrasound measurements is low in our setting (23).

Data analysis

Results are presented as mean (SD), median (interquartile range (IQR)) or proportions as appropriate. Non-normally distributed characteristics were logarithmically transformed prior to statistical analysis.

The relationships of arterial function parameters with c-IMT and plaque were assessed in multivariable linear and logistic regression models, respectively. These results were expressed as partial correlation coefficient (r) and odds ratio (OR) (95% confidence interval (CI)), respectively; corresponding p values were provided. As age, sex, race, heart rate, body height, body weight and brachial mean blood pressure are established potential confounders in the present context (5,9,10), these characteristics were entered into each of the initial models. Subsequently, other baseline characteristics (see Table 1) that had bivariate associations with the arterial function variables were additionally entered as potential confounders in fully adjusted models on atherosclerosis.

Receiver operator characteristic (ROC) curve analysis was implemented when arterial function measures were consistently related to atherosclerotic plaque. We calculated the Youden index and applied Bayes' theorem.

Statistical analysis was performed using IBM SPSS statistics program (version 23.0, IBM, USA). A p-value of ≤ 0.05 was considered significant. This included the construction of a ROC curve.

Results

Patient characteristics

Recorded patient characteristics are given in Table 1. Mean (SD) age and RA duration were 58.4 (11.7) and 16.0 (10.1) years, respectively. Hypertension, dyslipidemia and diabetes were observed in 41.7%, 49.1% and 6.1% of the patients, respectively. Disease activity was overall mild with a mean (SD) DAS28 of 2.9 (1.7) and median (IQR) CDAI of 5 (1-13). All except 12 (7.4%) patients were using synthetic or biologic disease modifying agents. The median (IQR) pulse wave velocity was 8.1 (6.2-9.4) m/sec and 46.6% of the patients had plaque.

Associations of arterial function with atherosclerosis in 163 RA patients

Table 2 shows that in univariate analysis, pulse wave velocity (partial $r = 0.277$, $p = 0.001$), reflected wave pressure (partial $r = 0.276$, $p < 0.001$), central systolic blood pressure (partial $r = 0.334$, $p < 0.001$), central pulse pressure (partial $r = 0.313$, $p < 0.001$), peripheral pulse pressure (partial $r = 0.165$, $p = 0.04$) and forward wave pressure (partial $r = 0.298$, $p < 0.001$) were associated with C-IMT. After adjustment for established potential confounders, pulse wave velocity, peripheral pulse pressure and forward wave pressure were no longer related to c-IMT whereas augmentation index (partial $r = 0.125$, $p = 0.04$) became associated with c-IMT. In fully adjusted models, except for central pulse pressure (partial $r = 0.161$, $p = 0.05$), arterial function measures were unrelated to c-IMT.

In univariate analysis, 1 SD increase in pulse wave velocity (OR (95% CI) = 1.67 (1.13-2.48), $p = 0.01$), reflected wave pressure (OR (95% CI) = 1.82 (1.28-2.59), $p = 0.001$), central systolic blood pressure (OR (95% CI) = 1.75 (1.22-2.50), $p = 0.002$), central pulse pressure (OR (95% CI) = 2.13

(1.46-3.12), $p < 0.001$), peripheral pulse pressure (OR (95% CI) = 2.13 (1.46-3.12), $p < 0.001$) and forward wave pressure (OR = 1.78 (1.25-2.54), $p < 0.001$) were each associated with carotid plaque. Upon adjustment for established potential confounders, pulse wave velocity, central systolic blood pressure and forward wave pressure were no longer associated with carotid plaque whereas reflection magnitude (OR (95% CI) = 1.76 (1.14-2.71), $p = 0.01$) became related to plaque. In fully adjusted models, reflected wave pressure (OR (95% CI) = 2.54 (1.41-4.44), $p = 0.001$), reflection magnitude (OR (95% CI) = 1.84 (1.17-2.89), $p = 0.008$), central pulse pressure (OR (95% CI) = 1.89 (1.12-3.22), $p = 0.02$) and peripheral pulse pressure (OR (95% CI) = 2.09 (1.23-3.57), $p = 0.007$) were associated with plaque. These results are further illustrated in Figure 1. Upon additional adjustment for pulse wave velocity in the latter 4 models, reflected wave pressure (OR (95% CI) = 2.56 (1.45-4.52), $p = 0.001$), reflection magnitude (OR (95% CI) = 1.94 (1.22-3.09), $p = 0.005$), central pulse pressure (OR (95% CI) = 1.91 (1.11-3.29), $p = 0.02$) and peripheral pulse pressure (OR (95% CI) = 2.11 (1.22-3.65), $p = 0.008$) remained related to plaque. Also, upon additional adjustment for dyslipidemia, diabetes and smoking in the latter 4 models, reflected wave pressure (OR (95% CI) = 2.38 (1.35-4.20), $p = 0.003$), reflection magnitude (OR (95% CI) = 1.91 (1.19-3.06), $p = 0.007$), central pulse pressure (OR (95% CI) = 1.82 (1.08-3.09), $p = 0.03$) and peripheral pulse pressure (OR (95% CI) = 1.81 (1.12-3.00), $p = 0.01$) remained related to plaque.

Cardiovascular and biologic agents can influence arterial function (5,29). Therefore, we reassessed the reflected wave pressure-, reflection magnitude-, central pulse pressure- and peripheral pulse pressure–plaque relationships in the last 2 columns in Table II with consistent additional adjustment for the use of angiotensin converting enzyme inhibitors ($n=23$), angiotensin receptor blockers ($n=27$), calcium channel blockers ($n=32$), beta receptor blocking agents ($n=14$), statins, tumor necrosis factor- α inhibitors and abatacept. Reflected wave pressure (OR (95% CI) = 2.15 (1.20-3.84), $p = 0.01$), reflection magnitude (OR (95% CI) = 1.56 (0.99-2.47), $p = 0.05$), central pulse pressure (OR (95% CI) = 1.71 (0.99-2.94), $p = 0.05$) and peripheral pulse pressure (OR (95% CI) = 2.21 (1.26-3.89), $p = 0.006$) remained associated with carotid plaque. By contrast, when we reassessed the central systolic blood pressure-c-IMT relationship (column 6 and 7 in Table II) with

additional adjustment of cardiovascular and biologic agent use, the respective association was no longer significant (partial $r = 0.112$, $p = 0.2$).

Wave reflection, pressure pulsatility and carotid plaque in RA

In Table 3, wave reflection and pressure pulsatility measures were entered together in 4 different established confounder and cardiovascular and biologic agent use adjusted regression models for carotid plaque. Reflected wave pressure and reflection magnitude remained consistently associated with plaque. Central and peripheral pulse pressure were no longer significantly associated with plaque when entered together with wave reflection measures.

Wave reflection is related to carotid plaque in hypertensive and normotensive patients with RA

In Table 4, the established confounder adjusted associations of arterial function measures with C-IMT and plaque were determined in hypertensive and normotensive RA patients. Apart from pulse pressure amplification (partial $r = -0.261$, $p = 0.05$) in hypertensive patients, arterial function was not independently related to c-IMT. Central pulse pressure and peripheral pulse pressure were associated with plaque in hypertensive (OR (95% CI) = 2.31 (1.10-4.82), $p = 0.02$ and OR (95% CI) = 2.53 (1.14-5.58), $p = 0.02$, respectively) but not in normotensive RA patients. By contrast, reflective wave pressure and reflection magnitude were related to plaque in both hypertensive (OR (95% CI) = 2.91 (1.28-6.62), $p = 0.01$ and OR (95% CI) = 2.43 (1.02-5.81), $p = 0.04$, respectively) and normotensive RA patients (OR (95% CI) = 2.93 (1.18-7.28), $p = 0.02$ and OR (95% CI) = 1.82 (1.04-3.20), $p = 0.03$, respectively). Additional adjustment for cardiovascular and biologic agent use was deemed inappropriate as it would have translated into the application of over-fitted models.

Accuracy of reflected wave pressure in predicting plaque presence

In the foregoing analysis, reflected wave pressure comprised the arterial stiffness measure that was most consistently related to carotid plaque prevalence. As shown in Figure 2, the area under the curve (0.658) in receiver operator characteristic curve analysis for reflected wave pressure was significantly ($p = 0.001$) associated with plaque presence. Based on calculation of the Youden

index (30) and application of Bayes' theorem (31), the optimal cut-off value for reflected wave pressure in predicting plaque presence was 25 mm Hg with a corresponding sensitivity, specificity, positive predictive value and negative predictive value of 45.2%, 89.3%, 78.6% and 66.2%, respectively. In a univariate logistic regression model, a reflected wave pressure of > 25 mm Hg (OR = 6.30 (2.82-14.07), $p < 0.0001$) was associated with plaque. This relationship was strengthened after adjusting for established potential confounders (OR = 13.71 (4.24-44.34), $p < 0.0001$).

Relationships of arterial function with atherosclerosis in women with RA

Women comprised 79.1 % of RA patients in the present study. The impact of arterial function on cardiovascular disease can differ by gender (5,7). Table 5 gives the results of a sensitivity analysis on the potential established confounder and fully adjusted arterial function-carotid plaque relations amongst 135 participating women with RA. Reflected wave pressure, reflection magnitude and central pulse pressure remained strongly associated with plaque whereas the peripheral pulse pressure-plaque relationship was attenuated ($p = 0.06$ in the fully adjusted model). Upon additional adjustment for cardiovascular and biologic agent use, reflected wave pressure (OR (95% CI) = 2.32 (1.22-4.42), $p = 0.01$) and reflection magnitude (OR (95% CI) = 1.78 (1.10-2.87), $p = 0.01$) remained associated with carotid plaque; the central pulse pressure-carotid plaque relationship was no longer significant (OR (95% CI) = 1.81 (0.89-3.35), $p = 0.06$). None of the arterial function measures was independently associated with c-IMT (data not shown). A sensitivity analysis was not performed in men due to the small number ($n = 28$) involved.

Upon replacement of mean blood pressure by brachial systolic and diastolic blood pressure in the models (except for those on peripheral pulse pressure) in tables 2, 3, 4 and 5, the results were materially unaltered (data not shown).

Discussion

The present study shows that wave reflection as measured by reflected wave pressure and reflection magnitude is strongly associated with carotid artery plaque in patients with RA. These

relationships were independent of potential confounders as well as arterial stiffness and central and peripheral pulse pressure, and were found in both hypertensive and normotensive patients with RA and in a sensitivity analysis amongst women. These findings support our hypothesis that wave reflection represents atherosclerosis in RA.

Overall, reflected wave pressure and reflection magnitude were consistently associated with plaque presence in both univariate and multivariable regression models in the present study. Wave reflection explained the associations of central and peripheral pulse pressure with plaque (Table 3). In receiver operator characteristic curve analysis, reflected wave pressure was also related to plaque presence. At a cutoff value of 25 mmHg with a sensitivity, specificity, positive predictive value and negative predictive value of 45.2%, 89.3%, 78.6% and 66.2%, respectively; a reflected wave pressure of >25 mmHg was associated with plaque in univariate and adjusted analysis, respectively. Arterial plaque mostly contains a lipid core (32) and is indeed a marker of high atherosclerotic cardiovascular disease risk for which the use of cardiovascular drugs including lipid lowering agents is indicated (15,33). The role of wave reflection in cardiovascular disease is increasingly documented in non-RA persons (5,7-10). This together with our current results suggests that wave reflection can contribute to cardiovascular disease risk and its stratification beyond brachial pressures and other risk factors in RA.

We recently reported that pulse wave velocity was associated with c-IMT and plaque in univariate but not confounder adjusted analysis among 194 Spanish RA patients (19). Additionally, the augmentation index was consistently unrelated to c-IMT and plaque in the latter investigation (19). Although our current results validate these findings, the potential impact of arterial stiffness and wave reflection as assessed by the augmentation index requires further elucidation in future larger and longitudinal studies.

In contrast to arterial plaque, intima-media thickening mostly represents vascular remodelling in response to blood pressure rather than atherosclerosis (34). C-IMT is particularly related to stroke (35) and its risk factors (36). In the present study, we found univariate associations of arterial stiffness and measures of wave reflection and pressure pulsatility with c-IMT but these relationships became insignificant after adjustment for potential confounders.

Arterial stiffening increases wave reflection and both of these arterial properties enlarge pulse pressure with a higher systolic and lower diastolic blood pressure (5). Despite these interlinking mechanisms, arterial stiffness, wave reflection and pressure pulsatility are also each determined by different factors, represent disparate aspects of arterial function and can contribute independently to cardiovascular risk (5). Our current findings are congruent with these previously reported observations. Additionally, we recently reported differential effects of traditional cardiovascular risk factors and disease characteristics on arterial stiffness, wave reflection and pressure pulsatility in patients with RA (12). Further mechanistic studies on arterial function in RA are clearly indicated.

The European Society of Hypertension/European Society of Cardiology guidelines for the management of arterial hypertension currently recommend determining arterial stiffness by pulse wave velocity in the identification of patients with target organ damage (37). It is therefore noteworthy that in contrast to other arterial function parameters, both reflected wave pressure and reflection magnitude were associated with carotid plaque in not only hypertensive but also normotensive patients with RA in the current status. This suggests that wave reflection may enhance cardiovascular risk and its stratification irrespective of hypertension status in RA.

Strikingly, peripheral pulse pressure was as strongly related to plaque presence as central pulse pressure in this RA investigation. In this regard, Dart and colleagues (38) previously reported that peripheral pulse pressure but not the carotid augmentation index and systemic arterial compliance predict incident cardiovascular events in elderly women with hypertension. In elderly compared to younger persons, brachial blood pressure more accurately estimates central pressure as pulse pressure amplification is reduced. This likely explains the equally strong association of brachial and central pulse pressure with plaque prevalence in the present context (38). Seventy-nine percent of our RA patients were women with a mean age of 58 years.

Our findings have treatment implications. Among antihypertensive agents, angiotensin converting enzyme inhibitors (ACEI), angiotensin receptor blockers (ARB) and calcium channel blockers reduce wave reflection (39). By contrast, beta blockers can increase wave reflection and the respective effect of diuretics is neutral (39). Hypertension is frequent and its management reportedly inadequate in RA (15). The 2015/2016 update of the European League Against

Rheumatism recommendations for cardiovascular disease risk management in patients with inflammatory joint diseases no longer advocate the preferred use of ACEI or ARB in the treatment of hypertension (15). Our current results together with previously reported evidence support ACEI, ARB or calcium channel blocker based first line treatment for hypertension in RA. In this regard, ACEI or ARB use is also associated with enhanced beta cell function in RA (40). Notably, Sveaas and colleagues (41) recently reported that high intensity endurance and strength exercise during 3 months results in significant decreases in pulse wave velocity as well as augmentation index in patients with axial spondyloarthritis. A recent meta-analysis revealed that aerobic but not resistance exercise reduces arterial stiffness and wave reflection in non-RA subjects (42). The effect of different exercise modalities on arterial function in patients with inflammatory joint disease requires further investigation. Resistance exercise is particularly indicated for the management of lean body mass loss associated with RA (43). Finally, tumour necrosis factor- α inhibition decreases pulse wave velocity whereas its effects on the augmentation index are inconsistent in RA (44). The influence of disease modifying agents on wave reflection as assessed in separation analysis has not been reported in RA.

Recently, Ikdahl and colleagues (45) reported an association between pulse wave velocity and incident cardiovascular events ($n = 10$) in RA. In contrast to wave reflection and pressure pulsatility, arterial stiffness was not independently related to carotid atherosclerosis in our current and previous investigation (19). This suggests that the effects of arterial stiffness on cardiovascular disease risk in RA may be mediated by mechanisms other than enhanced atherosclerosis such as decreased coronary blood flow and increased left ventricular afterload, which predisposes to arrhythmias (5,46).

We assessed the associations of 9 different arterial function indices with carotid artery plaque and IMT in comprehensively adjusted regression models among a relatively large multi-ethnic group of RA patients. Relationships between arterial function and atherosclerosis may be bidirectional (1). The main limitation of this investigation is the lack of a control population. Accordingly, whether our findings are RA specific should be the subject of future studies. Also, our study design was cross-sectional and therefore precluded identifying cause-effect relationships.

In conclusion, wave reflection is associated with severe atherosclerosis in RA. The role of wave reflection as an assessment tool and potential therapeutic target in cardiovascular risk management among patients with RA merits further study.

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Table I. Recorded characteristics in 163 RA patients.

Demographic characteristics			
Age at study time, years	58.4 (11.7)	ACPA positive	71.4
Female gender	79.1	CDAI	5 (1-13)
White	67.5	DAS 28	2.9 (1.7)
Asian	19.0	ESR, mm/h	13 (4-26)
Black	10.4	CRP, mg/l	3.3 (1.2-8.0)
Mixed	3.1	Leukocyte count, n/nl	6.0 (2.1)
Lifestyle factors		Deformed joints, n	7 (1)
Exercise	31.3	Extra-articular manifestations	22.7
Alcohol use	30.1	HAQ-DI	0.375 (0.000-0.875)
Current smoking	9.8	Synthetic DMARD	
Anthropometry		Methotrexate	76.1
BMI, kg/m ²	26.7 (5.5)	Chloroquine	50.3
Waist circumference, cm	92 (14)	Leflunomide	40.5
Waist-hip ratio	0.88 (0.08)	Sulphasalazine	16.6
Metabolic risk factors		Tetracycline	16.0
Hypertension	41.7	Azathioprine	6.7
Systolic BP, mm Hg	129 (15)	Current synthetic DMARD, n	2.1 (1.1)
Diastolic BP, mm Hg	81 (8)	Biological DMARD	
Total cholesterol, mmol/l	4.5 (1.0)	Abatacept	3.1
HDL cholesterol, mmol/l	1.68 (0.46)	TNF- α inhibitors	9.2
LDL cholesterol, mmol/l	2.5 (0.9)	NSAID	34.4
Triglycerides, mmol/l	1.0 (0.7-1.3)	Prednisone use	2.5
Total C – HDL C ratio	2.8 (0.8)	CKD-EPI, ml/min/1.73 m ²	99 (87-108)
Total C – HDL C ratio > 4	8.6	Heart rate, beats/min	73 (12)
Dyslipidemia	49.1	Arterial function	
Diabetes	6.1	Pulse wave velocity, m/sec	8.1 (6.2-9.4)
Glucose, mmol/l	4.9 (0.7)	Augmentation index, %	31 (11)
Insulin, μ U	6.4 (4.6-9.0)	Reflected wave pressure, mm Hg	21 (8)
HOMA-IR, μ U.mmol/ml.l	1.40 (0.96-1.98)	Reflection magnitude	76 (23)
Cardiovascular agent use		Central SBP, mm Hg	126 (15)
Antihypertensives	41.1	Central DBP, mm Hg	84 (9)
Statins	41.7	Central pulse pressure, mm Hg	42 (13)
Ezetimibe	11.7	Peripheral pulse pressure, mm Hg	48 (13)
Oral glucose-lowering agents	2.5	Pulse pressure amplification	1.20 (1.15-1.31)
Insulin, μ U/ml	1.8	Forward wave pressure, mm Hg	29 (9)
RA characteristics		Carotid atherosclerosis	
Disease duration, years	16.0 (10.1)	Mean IMT, mm	0.655 (0.137)
RF positive	74.2	Plaque	46.6

Continuous variables are expressed as mean (SD), median (interquartile range) or proportions as appropriate. RA: rheumatoid arthritis; BMI: body mass index; BP: blood pressure; HDL: high-density lipoprotein; LDL: low-density lipoprotein; C: cholesterol; HOMA-IR: Homeostatic Model Assessment of insulin resistance; RF: rheumatoid factor; ACPA: anti-citrullinated protein antibody; CDAI: Clinical disease activity score; DAS28: Disease Activity Score in 28 joints; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; HAQ-DI: Stanford Health Assessment Questionnaire disability index; DMARD: disease modifying anti-rheumatic drugs; TNF- α : tumour necrosis factor- α ; NSAID: non-steroidal anti-inflammatory drugs; CKD-EPI: Chronic Kidney Disease Epidemiology Collaboration; SBP: systolic blood pressure; DBP: diastolic blood pressure; IMT: mean intima-media thickness.

Table II. Relationships of arterial function measures with C-IMT and plaque in 163 RA patients.

	Univariate model		C-IMT Known confounder ¹ adjusted model		Fully adjusted ² multivariate model		Univariate model (for 1 SD increase)		Plaque Known confounder ¹ adjusted model (for 1 SD increase)		Fully adjusted ² Model (for 1 SD increase)	
	r	P	Partial r	p	Partial r	p	OR (95% CI)	p	OR (95% CI)	p	OR (95% CI)	p
PWV ³	0.277	0.001	-0.051	0.6	-0.045	0.6	1.67 (1.13-2.48)	0.01	1.11 (0.74-1.65)	0.6	1.03 (0.66-1.62)	0.9
AI ⁴	0.017	0.8	0.125	0.04	0.128	0.1	1.05 (0.76-1.44)	0.8	1.56 (0.98-2.48)	0.06	1.56 (0.94-2.70)	0.08
RWP ⁵	0.276	<0.001	0.128	0.04	0.128	0.1	1.82 (1.28-2.59)	0.001	2.50 (1.44-4.35)	0.001	2.54 (1.41-4.44)	0.001
RM ⁶	0.043	0.6	0.079	0.2	0.105	0.2	1.17 (0.89-1.53)	0.2	1.76 (1.14-2.71)	0.01	1.84 (1.17-2.89)	0.008
CSBP ⁷	0.334	<0.001	0.169	0.006	0.161	0.05	1.75 (1.22-2.50)	0.002	1.72 (0.98-3.01)	0.06	1.54 (0.85-2.79)	0.1
CPP ⁸	0.313	<0.001	0.129	0.04	0.131	0.1	2.13 (1.46-3.12)	<0.001	1.96 (1.18-3.26)	0.009	1.89 (1.12-3.22)	0.02
PPP ⁹	0.165	0.04	-0.026	0.7	-0.066	0.4	2.13 (1.46-3.12)	<0.001	1.95 (1.21-3.13)	0.006	2.09 (1.23-3.57)	0.007
PPA ¹⁰	-0.074	0.4	-0.094	0.1	-0.114	0.2	1.05 (0.76-1.43)	0.8	0.81 (0.45-1.46)	0.5	0.65 (0.34-1.27)	0.2
FWP ¹¹	0.298	<0.001	0.072	0.3	0.078	0.3	1.78 (1.25-2.54)	<0.001	1.31 (0.87-1.98)	0.2	1.27 (0.82-2.00)	0.3

Associations were determined in multivariable linear and logistic regression models for C-IMT and plaque, respectively. Known confounders¹ comprised age, sex, race, body height, body weight, heart rate and mean blood pressure. Significant relationships are shown in bold. C-IMT: carotid intima-media thickness; SD: standard deviation; OR: odds ratio; CI: confidence interval; PWV: pulse wave velocity; AI: augmentation index; RWP: reflected wave pressure; RM: reflection magnitude; CSBP: central systolic blood pressure; CPP: central pulse pressure; PPP: peripheral pulse pressure; PPA: pulse pressure amplification; FWP: forward wave pressure.

Variables additionally included in the different fully adjusted models² were:

³Waist-hip ratio, total cholesterol-HDL ratio, extra-articular manifestations and statin, tumour necrosis factor- α inhibitor and abatacept therapy

⁴Total cholesterol and HDL cholesterol concentrations, homeostatic model assessment for insulin resistance and tumour necrosis factor- α inhibitor therapy

⁵Number of synthetic disease modifying agents currently used

⁶HDL cholesterol concentrations, and tumour necrosis factor- α inhibitor and leflunomide therapy

⁷Total cholesterol-HDL cholesterol ratio and statin and chloroquine therapy

⁸Total cholesterol-HDL cholesterol ratio and statin and chloroquine therapy

⁹Total cholesterol-HDL cholesterol, C-reactive protein concentrations and statin, tetracycline and chloroquine therapy

¹⁰Waist-hip ratio, total cholesterol and glucose concentrations, and leflunomide and tumour necrosis factor- α inhibitor therapy

¹¹Total cholesterol-HDL cholesterol ratio and statin therapy

Table III. Independent relationships of 1 SD increase in arterial function measures in combination with plaque in 163 RA patients

Model	OR (95% CI)	p
Model 1 ¹		
RWP	8.34 (1.55-44.77)	0.01
CPP	0.27 (0.06-1.24)	0.09
Model 2 ¹		
RWP	1.89 (0.99-3.65)	0.05
PPP	1.22 (0.71-2.09)	0.5
Model 3 ¹		
RM	1.61 (1.04-2.50)	0.03
CPP	1.55 (0.93-2.56)	0.09
Model 4 ¹		
RM	1.61 (1.04-2.49)	0.03
PPP	1.52 (0.96-2.39)	0.07

¹Adjusted for known confounders of age, sex, race, body height, body weight, heart rate and mean blood pressure as well as cardiovascular drug and biologic agent use. Significant associations are shown in bold. SD: standard deviation; RWP: reflected wave pressure; CPP: central pulse pressure; PPP: peripheral pulse pressure; RM: reflection magnitude

Table IV. Independent relationships of arterial function measures with C-IMT and plaque in hypertensive and normotensive patients with RA.

Normotensive patients (n=100)		C-IMT	Plaque	
Hypertensive RA patients (n=68)				
	Partial r	P	OR (95% CI)	p
PWV	-0.039	0.8	0.68 (0.29-1.63)	0.4
AI	0.250	0.06	1.41 (0.75-2.65)	0.3
RWP	0.157	0.2	2.91 (1.28-6.62)	0.01
RM	0.132	0.3	2.43 (1.02-5.81)	0.04
CSBP	0.242	0.07	2.38 (0.97-5.84)	0.06
CPP	0.160	0.2	2.31 (1.10-4.82)	0.02
PPP	-0.059	0.7	2.53 (1.14-5.58)	0.02
PPA	-0.261	0.05	1.78 (0.64-4.92)	0.3
FWP	0.058	0.7	1.54 (0.84-2.81)	0.2
Normotensive RA patients (n=95)				
PWV	-0.046	0.7	1.35 (0.83-2.20)	0.2
AI	0.096	0.5	2.16 (0.99-4.69)	0.05
RWP	0.167	0.1	2.93 (1.18-7.28)	0.02
RM	0.093	0.4	1.82 (1.04-3.20)	0.03
CSBP	0.196	0.07	1.32 (0.59-2.96)	0.5
CPP	0.162	0.1	1.96 (0.87-4.44)	0.1
PPP	-0.034	0.8	1.67 (0.84-3.33)	0.1
PPA	-0.025	0.8	0.44 (0.17-1.13)	0.09
FWP	0.130	0.2	1.12 (0.59-2.15)	0.7

Associations were determined in age, sex, race, body height, body weight, heart rate and mean blood pressure adjusted multivariable linear and logistic regression models for C-IMT and plaque, respectively.

Significant relationships are shown in bold. C-IMT: carotid intima-media thickness; SD: standard deviation; OR: odds ratio; CI: confidence interval; PWV: pulse wave velocity; AI: augmentation index; RWP: reflected wave pressure; RM: reflection magnitude; CSBP: central systolic blood pressure; CPP: central pulse pressure; PPP: peripheral pulse pressure; PPA: pulse pressure amplification; FWP: forward wave pressure.

Table V. Independent relationships of arterial function with carotid plaque in 135 women with RA.

	Known confounder ¹ adjusted multivariable model (for 1 SD increase)		Fully adjusted Multivariable model ² (for 1 SD increase)	
	OR (95% CI)	p	OR (95% CI)	p
PWV ³	1.33 (0.77-2.28)	0.3	1.19 (0.62-2.26)	0.6
AI ⁴	1.62 (0.98-2.66)	0.06	1.73 (0.97-3.10)	0.06
RWP ⁵	2.87 (1.51-5.47)	0.001	3.00 (1.54-5.69)	0.001
RM ⁶	1.80 (1.14-2.85)	0.01	1.91 (1.18-3.09)	0.009
CSBP ⁷	1.85 (0.97-3.53)	0.06	1.51 (0.75-3.10)	0.2
CPP ⁸	2.23 (1.23-4.04)	0.008	2.06 (1.11-3.82)	0.02
PPP ⁹	1.68 (1.03-2.73)	0.04	1.71 (0.99-2.97)	0.06
PPA ¹⁰	0.71 (0.35-1.44)	0.4	0.58 (0.26-1.29)	0.2
RWP ¹¹	1.34 (0.83-2.19)	0.3	1.26 (0.76-2.09)	0.4

Associations were determined in multivariable logistic regression models. Known confounders¹ comprised age, race, body height, body weight, heart rate and mean blood pressure. Significant relationships are shown in bold. SD: standard deviation; OR: odds ratio; CI: confidence interval; PWV: pulse wave velocity; AI: augmentation index; RWP: reflected wave pressure; RM: reflection magnitude; CSBP: central systolic blood pressure; CPP: central pulse pressure; PPP: peripheral pulse pressure; PPA: pulse pressure amplification; FWP: forward wave pressure.

Variables additionally included in a different fully adjusted models² were:

³Waist-hip ratio, total cholesterol-HDL cholesterol ratio, extra-articular manifestations and statin, tumor necrosis factor- α inhibitor and abatacept therapy

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⁹Total cholesterol-HDL cholesterol and C-reactive protein concentrations and statin, tetracycline and chloroquine therapy

¹⁰Waist-hip ratio, total cholesterol and glucose concentrations, and leflunomide and tumor necrosis factor- α inhibitor therapy

¹¹Total cholesterol-HDL cholesterol ratio and statin therapy

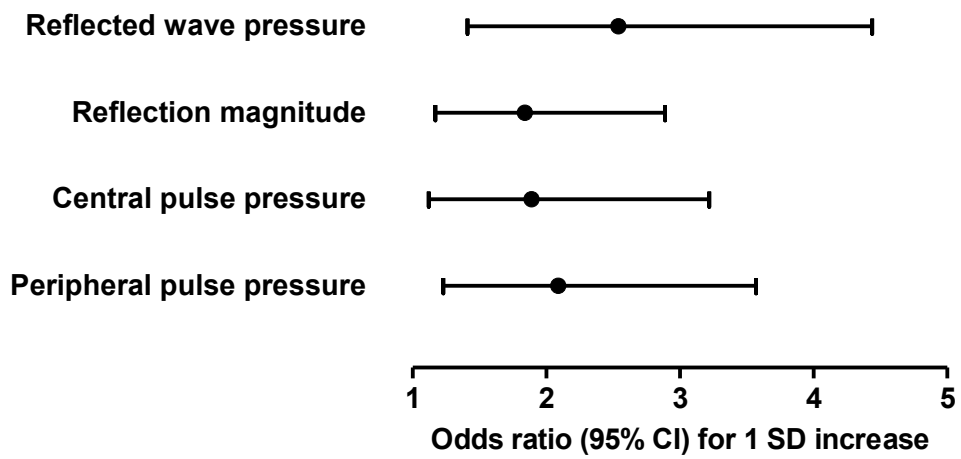


Figure I. Relationships of arterial function measures with carotid plaque.

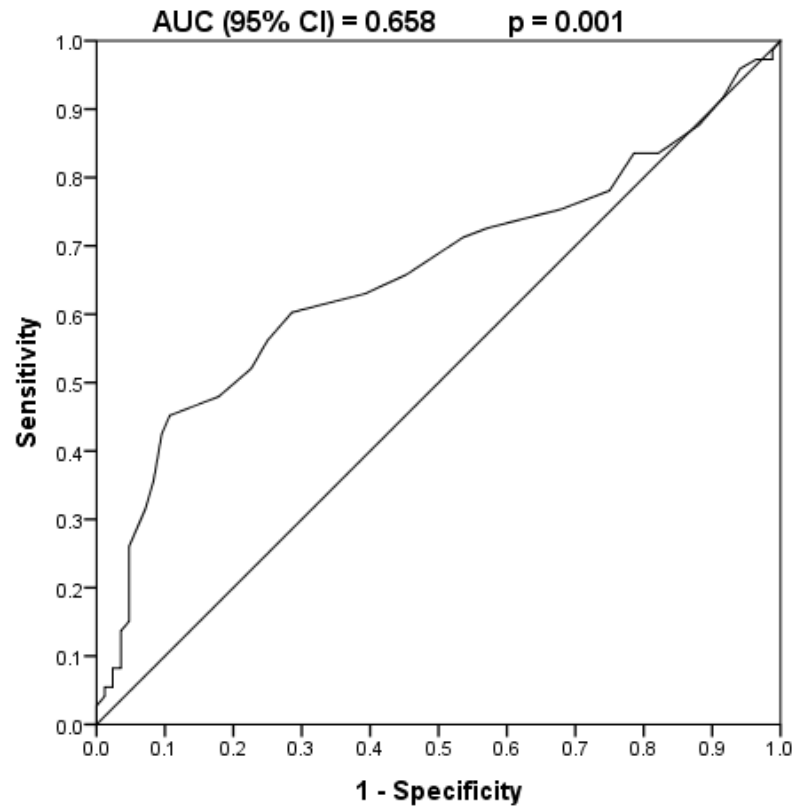


Figure II. Receiver operator characteristic curve showing the accuracy of reflected wave pressure in predicting plaque presence among 163 patients with RA.

Paper 2 - Arterial wave reflection and subclinical atherosclerosis in rheumatoid arthritis

Paper 2 showed that impaired arterial function and most notably arterial wave reflection, is strongly associated with atherosclerosis in patients with RA. Given (1) our findings in papers 1 and 2 together with (2) the recently reported beneficial effects of apelin on arterial function in non-RA subjects and (3) reduced apelin production in RA patients, we subsequently sought to determine the potential involvement of apelin in arterial function impairment among patients with RA.

Disease severity impacts the relationship of apelin with arterial function in patients with rheumatoid arthritis

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Abstract

Introduction: Apelin can improve arterial function by enhancing the expression of endothelial nitric oxide synthase but this effect depends markedly on endothelial integrity. We hypothesized that inflammation influences the potential impact of apelin on arterial function in rheumatoid arthritis (RA).

Methods: We assessed the associations of apelin concentrations with arterial stiffness (pulse wave velocity), wave reflection (augmentation index, reflected wave pressure and reflection magnitude) and pressure pulsatility (central systolic pressure (CSP), central pulse pressure (CPP), peripheral pulse pressure (PPP), pulse pressure amplification (PPamp) and forward wave pressure (Pf)) among 170 RA patients without cardiovascular disease.

Results: In multivariable regression models, apelin concentrations were not independently associated with arterial function measures ($p \geq 0.15$) in all patients. Inflammation markers were not consistently associated with apelin levels but joint deformity counts, Disease Activity Score in 28 joints (DAS28) and erythrocyte sedimentation rate (ESR) impacted apelin-pressure pulsatility relations (interaction $p \leq 0.05$). In stratified analysis, apelin was associated with CSP (partial $r = -0.33$, $p = 0.01$), CPP (partial $r = -0.26$, $p = 0.04$), PPamp (partial $r = 0.27$, $p = 0.03$) and Pf (partial $r = -0.33$, $p = 0.01$) in patients without but not with joint deformities; apelin was related to CSP (partial $r = -0.24$, $p = 0.05$) in those with a DAS28 joint < 2.8 (median value) (partial $r = -0.24$, $p = 0.05$) but not ≥ 2.8 , and to CSP (partial $r = -0.36$, $p = 0.003$) in those with an erythrocyte sedimentation rate < 13 mm/hr (median value) but not ≥ 13 mm/hr.

Conclusions: Apelin is associated with reduced pressure pulsatility in RA patients without but not with a high inflammatory burden. A loss of apelin protective effects on arterial function may contribute to the link between RA severity and cardiovascular risk.

Keywords Rheumatoid Arthritis Arterial Function Arterial Stiffness Wave reflection
Pressure Pulsatility Apelin

Introduction

Apelin is an endogenous peptide that acts on the APJ receptor [1-3]. The APJ receptor is located in the vascular endothelium and smooth muscle cells. Apelin is produced predominantly by endothelial cells and adipocytes. Amongst different identified apelin isoforms, apelin-13 is most strongly involved in the regulation of the cardiovascular system.

Enhancement of the apelin/APJ receptor signaling pathway has a range of potential beneficial effects on vascular and cardiac function [1,2,4]. Indeed, apelin administration increases endothelial nitric oxide synthase activity and expression and counteracts angiotensin II receptor induced vasoconstriction. Apelin thereby causes vasodilation and reduces blood pressure. Notably in the present context, these effects are markedly dependent on endothelial integrity [5-9]. Apelin treatment increases cardiac contractility by enhancing myofilament sensitivity to calcium [10] and activating kinase C and extracellular signal-regulated kinase $\frac{1}{2}$ [11]. Apelin decreases foam cell formation [12] and antagonizes angiotensin II induced atherosclerosis in ApoE knockout mice [13]. Apelin was recently shown to inhibit platelet function and thereby having antithrombotic effects [14]. Further, aerobic exercise training induces increased apelin levels that are associated with enhanced nitrite/nitrate concentrations and reductions in arterial stiffness [15]. Polymorphisms of the apelin gene are associated with changes in arterial stiffness [16].

It was recently established that the apelin receptor is additionally activated by a second ligand called Elabela or Toddler (gene name now Apela), which is present in human plasma and cardiovascular tissue [17]. This peptide has cardiovascular actions similar to apelin [17].

Arterial stiffness, increased wave reflection and pressure pulsatility comprise arterial properties that each predict cardiovascular events independent of traditional cardiovascular risk factors [18]. A prevailing paradigm is that increases in the propagation speed of the aortic pressure wave contribute to a faster return of the reflected wave. Enhanced arterial stiffness and wave reflection thereby increase systolic blood pressure and reduce diastolic blood pressure. The consequently enlarged pulse pressure, which is further determined by episodic cardiac contractility [19], increases left ventricular workload and reduces coronary blood flow. These hemodynamic effects result in increased cardiovascular event risk.

Rheumatoid arthritis (RA) enhances the risk of cardiovascular events and mortality to a similar extent as diabetes [20,21]. Systemic inflammation is the most implicated risk factor for the increased cardiovascular risk in RA [20,21]. The mechanisms whereby inflammation enhances cardiovascular event rates in RA await further elucidation. In this regard, patients with RA experience increased arterial stiffness, wave reflection and central pressures [22,23]. Tumor necrosis factor- α inhibition reduces arterial stiffness in RA [24]. Traditional cardiovascular risk factors and disease characteristics are independently associated with impaired arterial function [25] and arterial stiffness was recently shown to associate with incident cardiovascular event rates in RA [26].

Apelin levels are reportedly decreased in patients with RA [27]. We recently documented that apelin concentrations are associated with decreased atherosclerosis and arterial plaque vulnerability mediator levels in RA [28]. Herein, we explored the potential impact of apelin on arterial stiffness, wave reflection and pressure pulsatility in patients with RA. RA patients experience endothelial dysfunction and activation that are related to systemic inflammation and reduced upon disease activity suppression [29-32]. Therefore, we hypothesized that inflammation status influences the relationships of apelin concentrations with arterial function in RA.

Materials and methods

Patients

One hundred and seventy consecutive patients (142 women; 28 men) (112 white; 32 Asian; 22 black and 4 of mixed ancestry) that met the 2010 American College of Rheumatology/European League Against Rheumatism classification criteria for RA [33] and had no established cardiovascular disease were recruited at Milpark Hospital, Johannesburg, South Africa. The study was approved by the University of Witwatersrand Human (Medical) Research Ethics Committee (approval number: M06-07-33; protocol number: M120562) and all patients gave written informed consent.

Baseline characteristics

Baseline recorded characteristics included demographic variables, lifestyle factors, anthropometric measures, metabolic cardiovascular risk factors, RA features and cardiovascular and antirheumatic drug use. Disease activity was assessed by the Disease Activity Score in 28 joints (DAS28) and physical function by the Stanford Health Assessment Questionnaire disability index. Erythrocyte sedimentation rate (ESR) and leukocyte count was measured according to a standard laboratory protocol. C-reactive protein and insulin concentrations were quantified using immunoturbidimetry and a chemiluminescent particle immunoassay (Abbott Laboratories, Abbott Park, IL 60064 USA), respectively. Rheumatoid factor (RF) and anti-citrullinated protein antibody (ACPA) status were determined. Joint deformity counts and extra-articular manifestations (current evaluation and record review) were recorded.

Hypertension was diagnosed in the presence of an average systolic blood pressure of ≥ 140 and/or diastolic blood pressure of ≥ 90 mmHg and/or use of anti-hypertensive medications. Dyslipidemia was defined when the atherogenic index (total cholesterol-high density lipoprotein cholesterol ratio) was >4 [20] and/or lipid lowering medications were employed. The Homeostasis Model Assessment of insulin resistance (HOMA-IR) was calculated and diabetes was diagnosed in patients with a fasting plasma glucose of ≥ 7 mmol/l and/or those using glucose lowering agents. The glomerular filtration rate was estimated by the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation.

Arterial function measurements

Central arterial function was determined by radial applanation tonometry, as previously reported [34]. Pulse wave velocity and the peripheral wave form was measured using a high-fidelity SPC-301 micromanometer (Millar instrument, Inc., Houston, Texas), interfaced with a computer utilizing SphygmoCor software version 9.0 (AtCor Medical Pty.Ltd., West Ryde, New South Wales, Australia). After resting in the supine position for 15 minutes, the brachial blood pressure was firstly taken manually (by auscultation) for the purpose of calibrating the arterial waveforms to follow. Ten consecutive arterial waveforms (heart beats) were then recorded at the radial, femoral

and carotid artery pulses. Inconsistent waveforms were discarded when they differed by more than 5%, or if the overall amplitude of the pulse wave signal was less than 80mV. The peripheral pressure waveform was converted into a central aortic waveform, using a validated generalized transfer function incorporated in the SphygmoCor software. Central systolic pressure was derived from the aortic waveform. Central aortic pulse pressure was calculated from the difference between central systolic and diastolic pressure. Pulse pressure amplification represents the ratio between the peripheral (radial) pulse pressure and central aortic pulse pressure. The augmentation index is expressed as the ratio of the augmented pressure (difference between the second and first systolic shoulder) divided by the central pulse pressure x 100. SphygmoCor software separates the aortic waveform into forward and backward travelling components, by employing a modified triangular waveform technique. Reflected wave magnitude was calculated as the (reflected wave amplitude/forward wave amplitude) x 100.

Aortic pulse wave velocity was determined from sequential waveforms measurements at carotid and femoral sites by dividing the distance the wave travels by the transit time, as previously described [34]. To calculate the transit time (time delay) in pulse waves between the carotid and femoral sites, an electrocardiograph-derived R wave was utilized as fiducial point. Pulse transit time was calculated as the average of 10 sequential beats. The distance that the pulse wave travels was determined as the distance from the femoral sampling site to the suprasternal notch and the distance from the carotid sampling site to the suprasternal notch. The carotid-femoral pulse wave velocity was calculated as the distance between the carotid and femoral pulses in meters divided by the transit time in seconds.

All measurements were made by a single, experienced technician who was blinded to patient clinical data and cardiovascular risk factor profiles. Peripheral pulse pressure was recorded in all patients. Technically sound pulse wave velocity and wave reflection measurements were obtained in 156 and 163 patients, respectively. The intra-observer variability of tonometry measurements is low in our setting [34]. Among 28 patients that were evaluated on 2 occasions and 3 weeks apart, the intra-observer coefficients of variation ranged from 2.5% to 9.7% for the different arterial function measurements.

Echocardiography

As pressure pulsatility is determined by not only arterial characteristics but also episodic cardiac contractility [19] that can be influenced by apelin [10,11], we additionally performed echocardiographic measurements of left ventricular (LV) systolic function. We used commercially available equipment (Sonosite M-Turbo ultrasound, SonoSite® Inc., Bothell, WA, USA) with subjects in the left decubitus position.

LV dimensions were determined at both end systole and diastole, using two-dimensional directed M-mode echocardiography as recommended by the American College of Echocardiography [35].

Left ventricular end systolic volume (LVESV) was calculated using the equation $LVESV = [7.0 / (2.4 + \text{left ventricular end systolic diameter (LVESD)})] \times \text{left ventricular end systolic diameter (LVESD)}^3$, according to the Teichholz method [36].

Left ventricular ejection fraction (EF) was calculated as $[(LVEDV - LVESV) / LVEDV] \times 100$. Left ventricular (LVM) mass was derived using the Devereux formula [37] and indexed to height^{1.7} (LVMI).

Left ventricular relative wall thickness was calculated as $(LV \text{ diastolic posterior wall thickness (PWT)} \times 2) / LVEDD$ [38]. Midwall fractional shortening (FS_{mw}) was determined according to the following formula: $FS_{mw} = [LVEDD + (PWT_d + IVST_d) / 2] - (LVESD + (PWT_s + IVST_s) / 2) / [LVEDD + (PWT_d + IVST_d) / 2]$, where IVST denotes interventricular septum thickness and d diastole and s systole [39].

Echocardiography measurements were performed by two experienced operators that were blinded to the clinical data and cardiovascular disease risk factor profiles of patients. All echocardiographic images were reviewed by a single experienced operator. Intra- and inter-observer studies were conducted on 20 individuals. For the inter-observer variability, the Pearson's correlation coefficients for LVEDD, septal wall thickness and PWT were 0.71, 0.84 and 0.81, respectively ($p < 0.0001$ for all), and the coefficient of variation for LVEDD, septal wall thickness and PWT were 2.7%, 2.9% and 2.6%, respectively. There were no significant differences between the measures on unpaired t-tests ($p > 0.2$ for all). For the intra-observer variability, the Pearson's correlations for

repeat measures for observer one were 0.87, 0.76, and 0.98 and for observer two were 0.80, 0.88 and 0.89 for LVEDD, septal wall thickness and PWT, respectively (all $p < 0.001$); the respective coefficients of variation ranged between 1.4% and 4.7% for the one observer and 1.4% and 2.6% for these measures. There were no differences in the repeat measures on paired t-tests for either observer ($p > 0.2$ for all).

Apelin concentrations

The 77-preproapelin molecule is cleaved into shorter peptides including the post-translationally modified pyroglutamyl [Pyr¹] apelin-13, which is the most abundant isoform in the human cardiovascular system [40]. We determined apelin concentrations on one occasion, i.e. at the time of the study, using a solid-phase sandwich ELISA (RayBiotech Inc.; QuantikineHS). This test was designed to detect all active C-terminal apelin fragments including apelin-13, -31, -28 and apelin-36 without distinguishing between different apelin isoforms. The lower detection limit was 0.1ng/ml and the inter- and intra-assay coefficients of variation were <10% and <15%, respectively. A recovery experiment is included in the testing kit.

Data analysis

Continuous variables were expressed as mean (SD) or median (interquartile range (IQR)) as appropriate, and categorical variables as percentages. Logarithmic transformation was applied prior to statistical analysis when data were non-normally distributed.

Associations of baseline characteristics with apelin concentrations were evaluated in age at disease onset, gender and race adjusted linear regression models.

Relationships of apelin concentrations with measures of arterial function were initially determined in potential confounder ($p \leq 0.15$ for association in previous analysis) adjusted models. Body height, heart rate and systolic and diastolic blood pressure are further established confounders in the context of arterial function [25], and were therefore included as additional independent variables in subsequent models.

The impact of RA characteristics (see Table 1) on apelin-arterial function relationships was assessed using interaction analysis. Stratified analysis was subsequently performed when indicated (interaction $p < 0.05$); subgroups were determined using the median value when appropriate. Finally, significant relationships between apelin concentrations and arterial function in the foregoing analysis were re-evaluated in models with additional adjustment for cardiac contractility measures.

We utilized the IBM SPSS statistics program (version 23.0 IBM, USA) and significance was set at $p \leq 0.05$.

Results

The recorded characteristics are given in Table 1. Mean (SD) age was 57.8 (12.1) years and median (IQR) RA duration 14.5 (9.0-22.0) years. Dyslipidemia, hypertension and diabetes were present in 47.1%, 41.8% and 5.9%, respectively. Disease activity was overall mild with a mean (SD) DAS28 of 2.8 (1.7); RF and ACPA tested positive in 74.7% and 70.0% of the patients, respectively. The median (IQR) CRP was 3.1 (1.2-7.9) mg/l and 50% of the patients had at least one deformed joint. All except 15 patients employed synthetic disease modifying agents and 19 a biologic agent.

The median (IQR) left ventricular mass and mean (SD) relative wall thickness was 51.6 (41.7-68.9) g/m^{1.7} and 0.44 (0.08) mm, respectively. The majority of patients (74%) experienced an ejection fraction of $\geq 50\%$. The median (IQR) pulse wave velocity was 7.3 (6.2-9.3) m/sec and the mean (SD) peripheral and central pulse pressure were 47 (13) mm Hg and 42 (14) mmHg, respectively. The median (IQR) apelin concentration was 232 (149-339) pg/ml. In a previous study in which the same ELISA test was used as in our investigation [41], the median [IQR] apelin concentration was higher at 570 [415-680] pg/ml in healthy control subjects. This finding together with our current results support the notion that apelin levels are indeed reduced in RA patients as recently reported by Di Franco and colleagues [27].

Associations of baseline characteristics with apelin concentrations

Age at disease onset (partial $r=-0.20$, $p=0.01$) but not age at the time of the study (partial $r=-0.11$, $p=0.18$) was associated with apelin concentrations. Men and women had similar apelin concentrations (median (IQR)=206 (154-278) and 242 (148-354), respectively ($p=0.4$)). Other patient characteristic-apelin concentration relations (at $p \leq 0.15$) in age at disease onset, gender and race adjusted analysis are presented in Table 2. Based on these results, age at disease onset, deformed joints, RF status, DAS28, extra-articular manifestations, number of current DMARD used and HOMA-IR were included as potential confounders in subsequent analysis. Notably, disease activity as assessed by DAS28 as well as markers of inflammation including ESR, C-reactive protein concentrations and leukocyte counts were not significantly associated with apelin levels.

Associations of apelin concentrations with measures of arterial stiffness, wave reflection and pressure pulsatility in all patients

As shown in Table 3, apelin concentrations were inversely associated with reflected wave pressure ($p=0.01$), central systolic pressure ($p=0.01$) and central pulse pressure ($p=0.02$), and directly related to pulse pressure amplification ($p=0.003$) in univariate analysis.

When adjusting for potential confounders as identified in Table 2, apelin concentrations were inversely related to reflected wave pressure ($p=0.02$), central systolic blood pressure ($p=0.01$) and central pulse pressure ($p=0.01$), and directly related to pulse pressure amplification ($p=0.04$). Upon additional adjustment for established confounders in the present context (body height, heart rate and systolic and diastolic blood pressure), these associations were no longer significant.

Independent relationships of apelin concentrations with measures of pressure pulsatility in stratified analysis

Joint deformity count impacted apelin concentration-pressure relations (interaction $p=0.004$ for central systolic pressure, 0.01 for central pulse pressure, 0.01 for pulse pressure amplification and 0.0004 for forward wave pressure). Additionally, DAS28 and ESR influenced the apelin

concentration-central systolic blood pressure association (interaction $p=0.04$ and 0.05 , respectively). Other RA characteristics (Table 1) did not impact apelin-arterial function associations.

In age, gender and race adjusted stratified analysis, median (IQR) apelin concentrations tended to be lower in patients without compared to those with deformed joints (197 (127-328) pg/ml versus 274 (163-386) pg/ml, $p=0.06$); apelin levels did not differ significantly between patients with a DAS28 <2.8 and ≥ 2.8 (225 (149-345) pg/ml versus 271 (156-336) pg/ml, $p=0.2$) and between those with an ESR <13 mm/hr and ESR ≥ 13 mm/hr (221 (151-336) pg/ml versus 245 (149-346) pg/ml, $p=0.8$).

Table 4 shows the associations of apelin concentrations with pressure pulsatility measures in stratified analysis. Apelin concentrations were inversely associated with central systolic pressure (partial $r=-0.33$, $p=0.01$), central pulse pressure (partial $r=-0.26$, $p=0.04$) and forward wave pressure (partial $r=-0.33$, $p=0.01$), and directly related to pulse pressure amplification (partial $r=0.27$, $p=0.03$), in patients without but not with joint deformities. Apelin concentrations were inversely associated with central systolic pressures in patients with a DAS28 score of <2.8 (partial $r=-0.24$, $p=0.05$) but not ≥ 2.8 , and in those with an ESR of <13 mm/hr (partial $r=-0.36$, $p=0.003$) but not ≥ 13 mm/hr. Apelin concentrations were also associated with central pulse pressure, pulse pressure amplification and forward wave in patients with an ESR of <13 mm/hr but the respective relationships did not differ significantly from those found in patients with an ESR of ≥ 13 mm/hr (interaction $p > 0.05$). Upon additional adjustment for cardiovascular drugs (angiotensin converting enzyme inhibitors, angiotensin receptor blockers, calcium channel blockers, beta blockers and statins) in the models given in Table 4, the results were not materially altered (data not shown). Gender did not impact apelin concentration-pressure relations ($p=0.3$ to 1.0 for interactions).

Associations of apelin concentrations with measures of left ventricular mass and contractility

Apelin concentrations were not independently related to measures of left ventricular mass or contractility (data not shown). The ESR impacted on the relationships of apelin concentrations with left ventricular mass and relative wall thickness (interaction $p=0.05$ and 0.02 , respectively); in

stratified analysis, apelin concentrations were not related to either left ventricular mass nor relative wall thickness in patients with an ESR of <13 mm/hr and ≥ 13 mm/hr.

Relationships of apelin concentrations with arterial function are independent of left ventricular contractility

The associations of apelin concentrations with pressure pulsatility in stratified analysis (Table 4) was re-assessed upon additional adjustment for cardiac contractility markers. As given in Table 5, upon adjusting for stroke volume, associations of apelin concentrations with central systolic pressure (partial $r=-0.36$, $p=0.01$), central pulse pressure (partial $r=-0.28$, $p=0.03$), pulse pressure amplification (partial $r=0.30$, $p=0.01$) and forward wave pressure (partial $r=-0.37$, $p=0.004$) remained significant in patients without but not with joint deformities. Similarly, apelin remained related to central systolic pressure in those with a DAS28 <2.8 (partial $r=-0.24$, $p=0.05$) but not ≥ 2.8 and in those with an ESR of <13 mm/hr (partial $r=-0.36$, $p=0.003$) but not ≥ 13 mm/hr.

Upon adjusting for midwall fractional shortening, associations of apelin concentrations with central systolic pressure (partial $r=-0.36$, $p=0.01$), central pulse pressure (partial $r=-0.28$, $p=0.03$), pulse pressure amplification (partial $r=0.29$, $p=0.03$) and forward wave pressure (partial $r=-0.35$, $p=0.01$) remained significant in those without but not with joint deformities. Apelin concentration also remained related to central systolic blood pressure in those with a DAS28 <2.8 (partial $r=-0.25$, $p=0.05$) but not >2.8 and in those with an ESR <13 mm/hr, (partial $r=-0.37$, $p=0.003$) but not ≥ 13 mm/hr.

Lastly, upon adjustment for ejection fraction, the associations of apelin concentrations with central systolic pressure (partial $r=-0.36$, $p=0.01$), central pulse pressure (partial $r=-0.28$, $p=0.03$), pulse pressure amplification (partial $r=0.29$, $p=0.01$) and forward wave pressure (partial $r=-0.35$, $p=0.01$) remained significant in those without but not with joint deformities. Similarly, apelin concentration remained related to central systolic pressure in those with a DAS28 of <2.8 (partial $r=-0.25$, $p=0.05$) but not ≥ 2.8 and in those with an ESR <13 mm/hr (partial $r=-0.37$, $p=0.003$) but not ≥ 13 mm/hr.

Discussion

The main finding of the current study is that apelin concentrations are independently associated with reduced pressure pulsatility measures in RA patients without but not with a large current (DAS28 ≥ 2.8 or ESR ≥ 18 mm/h) or cumulative (presence of deformed joints) inflammatory burden. These results indicate that apelin may be involved in the link between inflammation and increased cardiovascular risk in RA.

After its discovery in 1998, systemic apelin administration was reported to decrease blood pressure by increasing endothelial nitric oxide synthase activity in animal models [1]. Apelin induced nitric oxide dependent arterial vasodilation was subsequently also documented in humans [4]. Endothelial nitric oxide diffuses in vascular smooth muscle cells where it activates the cyclic guanosine monophosphate-protein kinase G pathway [2]. This results in vasodilatation through vascular smooth muscle cell myosin light chain dephosphorylation and a reduction in cross bridges. However, in endothelium denuded vessels, apelin binds to APJ receptors that are located in smooth muscle cells [6,7]. Apelin thereby enhances myosin light chain phosphorylation that results in vasoconstriction [6,7]. Additionally, upon induction of endothelial dysfunction with asymmetric dimethylarginine (ADMA) or its analog molecule NG-nitro-L-arginine methyl ester (L-NAME), systemic apelin was found to cross the endothelial barrier and cause vascular smooth muscle cell myosin light chain phosphorylation with consequent vasoconstriction and increased blood pressure [5,8,9].

Increased systemic inflammation comprises the most implicated risk factor for endothelial dysfunction in patients with RA [29-32,42,43]. In the present investigation, besides cumulative inflammation as reflected by deformity joint count (lower apelin levels in patients without compared to with joint deformities), disease activity and inflammation markers were not significantly associated with apelin concentrations. However, the apelin-pressure pulsatility relations and hence presumably the effects of apelin on arterial function in RA, were significantly modulated by both current or cumulative inflammation status as represented by a high ESR and DAS28 or joint deformity count, respectively. Our results in the present study together with previously reported findings [5-9] suggest that among RA patients that experience high-grade current or cumulative

inflammation, endothelial nitric oxide dependent vasodilation induced by apelin is negated by vasoconstriction through simultaneous activation of APJ receptors in arterial smooth muscle cells. The potential modulating effect of systemic inflammation induced endothelial dysfunction on the impact of apelin in arterial function among RA patients merits further evaluation in future longitudinal and mechanistic studies.

Whereas apelin concentrations were associated with pressure pulsatility in RA, we did not find relationships between apelin levels and pulse wave velocity, a valid marker of arterial stiffness [18], and wave reflection measures. This reinforces the notion that different arterial function measures as assessed in this study, are determined by disparate factors [18,25].

In persons aged 60 years or more, peripheral pulse pressure predicts coronary heart disease risk better than systolic and diastolic blood pressure [44]. Pulse pressure is associated with endothelial dysfunction and both of these cardiovascular disease risk factors are dependent on nitric oxide bioavailability [45,46]. In the current study, apelin concentrations were associated with central but not peripheral pulse pressures in RA. In this regard, central pressures are lower than peripheral pressures but more strongly related to target organ damage involving the heart, kidneys and cerebral arteries as well as incident cardiovascular events than peripheral blood pressure [18].

Apelin can increase cardiac contractility [10,11], which contributes to pressure pulsatility [19]. This effect could therefore have impacted the apelin concentration-pressure pulsatility relations in the present investigation. Increased left ventricular contractility would however be expected to increase pressure pulsatility [19]. By contrast, we found that apelin levels were inversely associated with pressure pulsatility and not related to left ventricular systolic function in RA. Upon adjustment for cardiac contractility measures, the relationships of apelin concentrations with reduced pressure pulsatility were unaltered in this RA study.

In line with current recommendations on the management of RA, the majority of patients that participated in this study experienced adequate disease activity control. Our results illustrate that it may therefore become increasingly indicated to consider the use of interaction and stratified analysis in RA studies aimed at identifying potential mechanisms underlying the association of systemic inflammation with cardiovascular disease risk [47]. Accordingly, we recently also

reported that apelin concentrations are more consistently related to decreased atherosclerosis and plaque vulnerability mediator levels in RA patients without compared to those with high inflammatory markers [28]. Interestingly, using the same approach, Dimitroulos and colleagues documented very recently that ADMA as well as symmetric dimethylarginine may mediate the effects of systemic inflammation on impaired micro- and macrovascular function in patients with RA [43].

We performed a comprehensive assessment of arterial function and considered a wide range of potential confounders including echocardiographic measures of systolic function, in the data analysis. However, our study design was cross-sectional, which precludes drawing inferences on the direction of causality. Also, the effects of different apelin isoforms and particularly [Pyr1]-apelin-13, as well as Elabela or Toddler [17] on arterial function in patients with RA require exploration in future studies.

In conclusion, we documented that apelin concentrations are independently associated with reduced pressure pulsatility in RA patients with a low current or cumulative inflammatory burden. By contrast, apelin levels were unrelated to arterial function in patients with high inflammatory markers. A loss of protective effects on the vasculature by apelin may mediate systemic inflammation related increased cardiovascular disease risk in patients with RA.

Author contributions

SG, LT, AMEM and PHD conceived and designed the study. SG, CR, LT, AMEM and PHD collected the data. SG, AMEM and PHD performed the data analysis and interpretation. SG and PHD drafted the manuscript. CR, GRN and AJW contributed to revising the manuscript critically for important intellectual content. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The study was approved by the University of Witwatersrand Human (Medical) Research Ethics Committee (approval number: M06-07-33; protocol number: M120562). All patients gave written informed consent.

Conflict of interest

The authors declare that they have no conflicts of interest.

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Table 1 Recorded characteristics in 170 patients with RA

<i>Demographic characteristics</i>		RF positive	74.7
Age at study time, years	57.8 (12.1)	ACPA positive	70.0
Age at disease onset	41.8 (13.9)	DAS28	2.8 (1.7)
Female gender	83.5	ESR, mm/hr	13.0 (4.0-26.0)
White	65.9	CRP, mg/l	3.1 (1.2-7.9)
Asian	18.8	Leukocyte count, n/nl	5.3 (4.5-6.8)
Black	12.9	Deformed joints, n	1.0 (0 - 10.0)
Coloured	2.4	Extra-articular manifestations	21.2
<i>Lifestyle factors</i>		HAQ-DI	0.4 (0.0-0.9)
Exercise	32.4	<i>Synthetic disease modifying agents</i>	90.6
Alcohol use	31.2	Methotrexate	75.9
Current smoking	9.4	Chloroquine	51.2
<i>Anthropometry</i>		Leflunomide	39.4
BMI, kg/m ²	27.0 (5.5)	Sulphasalazine	15.9
Waist circumference, cm	91.9 (14.0)	Tetracycline	15.9
Waist-hip ratio	0.88 (0.09)	Azathioprine	7.1
<i>Metabolic risk factors</i>		Current DMARD, n	2.1 (1.1)
Hypertension	41.8	<i>Biological disease modifying agents</i>	11.2
SBP, mm Hg	128 (16)	Abatacept	2.9
DBP, mm Hg	81 (8)	TNF- α inhibitors	8.2
Total cholesterol, mmol/l	4.5 (1.1)	NSAID	35.3
HDL cholesterol, mmol/l	1.68 (0.46)	Prednisone use	2.4
LDL cholesterol, mmol/l	2.4 (0.9)	CKD-EPI, ml/min/1.73 ²	99.3 (86.1 -107.6)
Triglycerides, mmol/l	0.93 (0.70-1.25)	Heart rate	72.6 (11.9)
Total C - HDL C ratio	2.7 (2.3-3.2)	<i>Echocardiographic measures</i>	
Total C - HDL C ratio >4	8.2	LV mass, g/m ^{1.7}	51.6 (41.7-68.9)
Dyslipidemia,	47.1	Relative wall thickness, mm	0.44 (0.08)
Diabetes	5.9	Stroke volume, ml	72.3 (12.0)
Glucose, mmol/l	4.9 (4.5-5.1)	FSmw, mm	20.7 (6.8)
HOMA-IR, μ U.mmol/ml.l	1.4 (1.0-2.0)	Ejection fraction, %	58.8 (13.5)
<i>Cardiovascular agents</i>		<i>Arterial function measures</i>	
Antihypertensives	41.2	Pulse wave velocity, m/sec	7.3 (6.2-9.3)
Diuretics	22.4	Augmentation index, %	31 (11)
ACEI	15.3	Reflected wave pressure, mm Hg	21 (8)
ARB	15.9	Reflection magnitude, %	75 (23)
CCB	18.8	Central systolic pressure, mm Hg	126 (16)
BB	8.2	Central diastolic pressure, mm Hg	84(9)
Statins	40.6	Central pulse pressure, mm Hg	42 (14)
Ezetimibe	10.6	Pulse pressure amplification	1.2 (1.0-1.3)
OGLA	2.4	Peripheral pulse pressure, mm Hg	47 (13)
Insulin	1.8	Forward wave pressure, mm Hg	28 (9)
<i>RA characteristics</i>		Apelin concentration, pg/ml	232 (149-339)
Disease duration, years	14.5 (9.0-22.0)		

Dichotomous variables are expressed as proportions or percentages and continuous variables as mean (SD) or median (interquartile range). RA: rheumatoid arthritis; BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; HDL: high-density lipoprotein; LDL: low-density lipoprotein; HOMA-IR: Homeostasis Model Assessment of insulin resistance; ACEI: angiotensin converting enzyme inhibitors; ARB: angiotensin II receptor blockers; CCB: calcium channel blocker; BB: beta blockers; OGLA: oral glucose lowering agents; RF: rheumatoid factor; ACPA: anti-citrullinated protein antibody; CDAL: Clinical Disease Activity Index; DAS28: Disease Activity Score in 28 joints; ESR: erythrocyte sedimentation rate; CRP: C reactive protein; HAQ: Stanford Health Assessment Questionnaire Disability Index; DMARD: Disease modifying anti-rheumatic drugs; NSAID: non-steroidal anti-inflammatory drugs; CKD-EPI: Chronic Kidney Disease Epidemiology Collaboration; LV: Left ventricular; RWT: relative wall thickness; FSmw: midwall fractional shortening.

Table 2 Associations of baseline characteristics with apelin concentrations at p value ≤ 0.15

	Partial r	P value
Age at disease onset	-0.20	0.01
Deformed joints	0.19	0.02
RF positive	0.20	0.01
DAS28	0.12	0.13
Extra articular manifestations	0.13	0.10
Methotrexate	0.21	0.01
Leflunomide	0.12	0.14
Sulphasalazine	0.12	0.15
Number of DMARD	0.20	0.01
HOMA-IR	0.17	0.04

Associations were determined in age at disease onset, gender and race adjusted models. RF: rheumatoid factor; DAS28: Disease Activity Score in 28 joints; DMARD: disease modifying anti-rheumatic drugs; HOMA-IR: Homeostasis Model Assessment of insulin Resistance.

Table 3 Associations of apelin concentrations with arterial function in 170 patients with RA

		Arterial stiffness	Wave reflection			Pressure pulsatility				
		Pulse wave velocity	Augmentation index	Reflected wave pressure	Reflection magnitude	Central SP	Central PP	PP amplification	Peripheral PP ¹	Forward wave pressure
Univariate	r (p)	-0.01 (0.84)	-0.15 (0.06)	-0.19 (0.01)	-0.12 (0.11)	-0.19 (0.01)	-0.19 (0.02)	0.23 (0.003)	-0.12 (0.10)	-0.09 (0.24)
Adjusted ²	Partial r (p)	0.04 (0.69)	-0.13 (0.13)	-0.19 (0.02)	-0.07 (0.44)	-0.22 (0.01)	-0.22 (0.01)	0.17 (0.04)	-0.10 (0.26)	-0.15 (0.08)
Adjusted ³	Partial r (p)	0.07 (0.47)	-0.14 (0.87)	-0.07 (0.43)	0.09 (0.31)	-0.13 (0.15)	-0.12 (0.16)	-0.04 (0.64)	0.14 (0.11)	-0.12 (0.15)

Significant associations are shown in bold. SP: Systolic pressure; PP: pulse pressure

¹Systolic and diastolic blood pressure were replaced by mean blood pressure in models on peripheral pulse pressure.

²Adjusted for age at disease onset, gender, race, number of deformed joints, DAS 28, RF status, extra articular manifestations number of DMARDS, HOMA-IR (see Table 2)

³Additionally adjusted for height, systolic blood pressure, diastolic blood pressure and heart rate

Table 4 Independent associations of apelin concentrations with measures of pressure pulsatility in stratified analysis

	Central SP		Central PP		PP amplification		Peripheral PP ¹		Forward wave	
	Partial r	P value	Partial r	P value	Partial r	P value	Partial r	P value	Partial r	P value
Deformed joints										
<1	-0.33	0.01	-0.26	0.04	0.27	0.03	-0.08	0.52	-0.33	0.01
≥1	0.09	0.47	0.07	0.61	-0.08	0.54	0.04	0.78	0.11	0.09
DAS28										
<2.8	-0.24	0.05	-0.17	0.17	0.18	0.14	-0.02	0.86	-0.07	0.55
≥2.8	-0.02	0.91	-0.08	0.55	0.09	0.52	-0.13	0.34	-0.18	0.20
ESR										
<13 mm/hr	-0.36	0.003	-0.29	0.02	0.31	0.01	-0.06	0.61	-0.29	0.02
≥13 mm/hr	-0.90	0.50	-0.07	0.34	0.16	0.24	0.08	0.62	-0.10	0.30

Age at disease onset, gender, race, height, heart rate, systolic and diastolic blood pressure, deformed joints, DAS28, RF status, number of DMARD, HOMA-IR, and extra-articular manifestations were adjusted for in each model. Significant associations are shown in bold. SP: systolic pressure; PP: pulse pressure; DAS28: Diseases Activity Score in 28 joints; ESR: erythrocyte sedimentation rate.

¹Systolic and diastolic blood pressure were replaced by mean blood pressure in models on peripheral pulse pressure.

Table 5 Cardiac contractility independent associations of apelin concentration with measures of central pressure pulsatility in stratified analysis

		Central SP		Central PP		PP amplification		Peripheral PP ¹		Forward wave	
		Partial r	P value	Partial r	P value	Partial r	P value	Partial r	P value	Partial r	P value
Adding stroke volume to the model	Deformed joints										
	≤1	-0.36	0.01	-0.28	0.03	0.30	0.01	-0.11	0.37	-0.37	0.004
	>1	0.13	0.31	0.05	0.74	-0.10	0.42	0.10	0.44	0.27	0.15
	DAS28										
	≤2.8	-0.24	0.05	-0.17	0.18	0.17	0.14	0.00	0.99	-0.08	0.50
	>2.8	0.03	0.84	-0.05	0.72	0.09	0.52	-0.03	0.81	-0.15	0.30
	ESR										
Adding FSmw to the model	≤13 mm/hr	-0.36	0.003	-0.29	0.002	0.31	0.01	-0.04	0.78	-0.28	0.02
	>13 mm/hr	-0.06	0.68	-0.10	0.46	0.14	0.32	0.06	0.68	-0.12	0.37
Adding EF to the model	Deformed joints										
	≤1	-0.36	0.01	-0.28	0.03	0.29	0.03	-0.07	0.60	-0.35	0.01
	>1	0.10	0.46	0.08	0.56	-0.09	0.50	0.05	0.71	0.14	0.30
	DAS28										
	≤2.8	-0.25	0.04	-0.16	0.19	0.19	0.13	-0.004	0.97	-0.09	0.49
	>2.8	0.02	0.90	-0.07	0.63	0.07	0.61	-0.13	0.36	-0.16	0.26
	ESR										
Adding EF to the model	≤13 mm/hr	-0.37	0.003	-0.29	0.02	0.31	0.01	0.07	0.60	-0.31	0.02
	>13 mm/hr	-0.03	0.83	-0.11	0.46	0.16	0.26	0.06	0.68	-0.12	0.40
Adding EF to the model	Deformed joints										
	≤1	-0.36	0.01	-0.28	0.03	0.29	0.03	-0.07	0.59	-0.35	0.01
	>1	0.09	0.52	0.06	0.68	-0.07	0.63	0.05	0.69	0.12	0.36
	DAS28										
	≤2.8	-0.25	0.05	-0.16	0.20	0.19	0.14	-0.01	0.94	-0.08	0.50
	>2.8	0.003	0.98	-0.08	0.58	0.08	0.57	-0.13	0.36	-0.17	0.25
	ESR										
Adding EF to the model	≤13 mm/hr	-0.37	0.003	-0.30	0.02	0.32	0.01	0.08	0.53	-0.31	0.01
	>13 mm/hr	-0.01	0.92	-0.10	0.48	0.16	0.27	0.06	0.66	-0.11	0.43

Age at disease onset, gender, race, height, heart rate, systolic and diastolic blood pressure, deformed joints, DAS28, RF status, number of DMARD, HOMA-IR, and extra-articular manifestations were adjusted for in each model. Significant associations are shown in bold. SP: systolic pressure; PP: pulse pressure; DAS28: Diseases Activity Score in 28 joints; ESR: erythrocyte sedimentation rate; FSmw: midwall fractional shortening; EF: ejection fraction.

¹Systolic and diastolic blood pressure were replaced by mean blood pressure in models on peripheral pulse pressure.

Paper 3 - Disease severity impacts the relationship of apelin with arterial function in patients with rheumatoid arthritis

Paper 3 showed that apelin may protect against adverse proximal aortic modeling and impaired function in RA. Our analysis further revealed that a large current or cumulative inflammatory burden may abrogate this cardioprotective effect of apelin in RA. Apelin was previously also shown to reduce atherosclerosis extent and atherosclerotic plaque vulnerability in non-RA studies. Therefore, we subsequently explored the potential role of apelin in atherosclerosis in RA.



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Apelin concentrations are associated with altered atherosclerotic plaque stability mediator levels and atherosclerosis in rheumatoid arthritis



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ABSTRACT

Background and aims: Apelin-APJ signaling reduces cardiovascular disease (CVD) risk. In rheumatoid arthritis (RA), the atherosclerosis burden and plaque vulnerability to rupture are increased. We explored relationships between apelin concentrations and subclinical CVD in RA.

Methods: Apelin levels were measured in 235 (114 black, 121 white) RA patients. Associations between apelin concentrations and ultrasound determined carotid artery intima-media thickness (cIMT) and plaque, and levels of matrix metalloproteinase (MMP)-2 and -9 that mediate plaque stability and vulnerability respectively, were identified in confounder adjusted multivariate regression analysis.

Results: In all patients, apelin concentrations were directly associated with those of MMP-2 (β (SE) = 0.324 (0.112), p = 0.004) and inversely with those of MMP-9 (β (SE) = -0.239 (0.060), p = 0.000). Apelin concentration-subclinical CVD relations were influenced by population origin, RA disease activity, erythrocyte sedimentation rate (ESR) and interleukin (IL)-6 concentrations (interaction p = 0.001 to 0.04). Accordingly, the apelin-MMP-2 concentration relationship was reproduced in white (β (SE) = 0.367 (0.146), p = 0.01) but not black RA patients (β (SE) = 0.197 (0.220), p = 0.4), and only in those without (but not with) large erythrocyte sedimentation rates (β (SE) = 0.428 (0.143), p = 0.003) or interleukin-6 levels (β (SE) = 0.485 (0.288), p = 0.04). By contrast, the apelin-MMP-9 concentration relation was reproduced more consistently. Apelin levels were inversely related to cIMT in patients with RA remission or mild (β (SE) = -0.068 (0.033), p = 0.04) but not moderate or high disease activity (β (SE) = 0.015 (0.112), p = 0.7).

Conclusions: Apelin concentrations are associated with altered plaque stability mediator levels and atherosclerosis in patients with RA. These relations are partially dependent on population origin and systemic inflammatory status.

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1. Introduction

In 1993, O'Dowd et al. identified the APJ receptor as a member of the 7-transmembrane G-protein coupled receptors [1]. The APJ receptor shows closest sequence homology to the angiotensin II

type 1 receptor (AT1) but does not bind angiotensin II. Tatemoto et al. found that apelin was an endogenous ligand for the APJ receptor in 1998 [2]. Apelin and APJ mRNA are expressed in the central nervous system and various peripheral tissues with a particular preponderance for the cardiovascular system [3].

Apelin production is increased in hyperinsulinemia-associated obesities [4] and in obese diabetic but not obese non-diabetic subjects [5]. The increased apelin production in obesity likely represents a compensatory and adaptive mechanism aimed at forestalling insulin resistance and other obesity related cardiovascular

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disorders [6,7]. Indeed, apelin enhances glucose utilization through both insulin-dependent and -independent mechanisms [8].

Opposite to the effects of angiotensin II, apelin reduces vascular tone and reduces blood pressure through endothelial-dependent nitric oxide-mediated vasodilatation, and increases diuresis by inhibiting vasopressin release [3,9]. Statin therapy results in increased apelin levels that are associated with decreases in total and LDL cholesterol concentrations [10]. Apelin signaling was shown to block angiotensin II induced atherosclerosis by increasing nitric oxide production and inhibiting angiotensin II signaling [11]. Apelin increases cholesterol efflux in macrophages and inhibits foam cell formation through activation of class III PI3K and beclin-1 mediated autophagy [12]. In the elastase-induced abdominal aortic aneurysm (AAA) and inflammation model, apelin administration markedly reduces vascular wall macrophage infiltration and cytokine and chemokine expression, and diminishes AAA formation [13]. Amongst patients with coronary artery disease (CAD), apelin concentrations are decreased [14], inversely associated with the severity and acute phase of CAD [15]. In patients with carotid stenosis, apelin concentrations are reduced and associated with increased carotid plaque vulnerability, and atorvastatin-induced increases in apelin levels predict increased plaque stability [16]. Taken together, the APJ-apelin system reduces cardiovascular risk and represents a promising therapeutic target in cardiovascular disease (CVD) [3].

Different matrix metalloproteinases can either promote a net extracellular matrix destruction that results in atherosclerotic plaque vulnerability to rupture, or enhance fibrosis that translates into plaque stability [17,18]. In this regard, matrix metalloproteinase (MMP)-2 enhances vascular smooth muscle cell migration and proliferation, which is implicated in the formation of fibrous caps in plaques. MMP-2 concentrations are larger in stable compared to unstable plaques. In contrast, MMP-9 levels are larger in unstable compared to stable plaques among human subjects. MMP-9 enhances extracellular matrix destruction rather than fibrosis.

The enhanced risk of CVD in rheumatoid arthritis (RA) is well established [19]. RA patients experience an increased atherosclerotic burden and enhanced plaque vulnerability and vascular inflammation [20–24]. Systemic inflammation is strongly implicated in RA associated CVD, but the underlying mechanisms remain uncertain [19]. In this regard, apelin concentrations are reduced in patients with untreated early RA [25].

Our aim was to explore independent relationships of apelin concentrations with ultrasound determined carotid atherosclerosis and levels of matrix metalloproteinase (MMP)-2 and -9 as mediators of plaque stability and vulnerability respectively [17,18], in patients with RA.

2. Patients and methods

2.1. Patients

The local Ethics Committee approved the study (M06 07 33) and written informed consent was obtained from each participant. Two hundred and thirty-five (114 black, 121 white) sequential patients who met reported RA classification [26,27] were enrolled. Patients were recruited at the Charlotte Maxeke Johannesburg Academic Hospital ($n = 132$) and the Milpark Hospital ($n = 103$) in Johannesburg. For each recorded feature, missing data were below 5%.

2.2. Baseline characteristics and conventional metabolic risk factors

Previously reported methods were employed [28]. In short, height, weight, waist and hip circumference were measured. RA

activity was assessed by the Disease Activity Score in 28 joints (DAS28) and the Clinical Disease Activity Score (CDAI). We recorded joint deformity counts, rheumatoid factor (RF) and extra-articular manifestations [28].

The erythrocyte sedimentation rate (ESR), renal and liver function, hematological variables, lipids and glucose were determined. The Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation was calculated [29]. C-reactive protein (CRP) concentrations were assessed by immunoturbidimetry. A solid-phase sandwich enzyme-linked immunosorbent assay (ELISA) was used to quantify interleukin-6 (IL-6) concentrations [28]. Data on synthetic disease modifying agent and cardiovascular drug use were included.

We measured blood pressure, lipid and glucose concentrations. A systolic and diastolic blood pressure of ≥ 140 and/or ≥ 90 mmHg respectively, and/or antihypertensive drug use were considered to represent hypertension. A cholesterol/HDL ratio >4 indicated the presence of dyslipidemia [19]. Diabetes was diagnosed when the fasting plasma glucose concentration was ≥ 7 mmol/L or glucose lowering agents were employed [30].

2.3. Atherosclerosis

Two operators, one in the private and one in the public sector, performed carotid ultrasonography in accordance with reported recommendations [31]. The carotid intima-media thickness (cIMT) was measured by obtaining images over at least 1 cm of the distal common carotid arteries and 1 cm proximal to the carotid bifurcation; the longitudinal angle of approach, where both internal and external carotid artery branches are best seen, was employed [32]. Measurements were performed with high-resolution B-mode US (Image Point, Hewlett Packard and SonoCalc IMT, Sonosite Inc.), using linear array 7.5 MHz probes. The ultrasonography methodology used in private healthcare patients was reported [33]. In public healthcare patients, specialized software was used that provides semi-automated border detection and markedly reproducible data [32]. The reported cIMT in this study is the mean of 2 values comprising those obtained at each distal common carotid artery. Plaque represents a luminal protrusion of ≥ 0.5 mm or 50% of the nearby intima-media thickness or an intima-thickness of >1.5 mm [31]. Both operators were blinded to cardiovascular risk factor profiles. Repeat ultrasonographic measurements were made by the 2 operators using their respective ultrasound machines on a group of 23 patients from either healthcare sector. The intra-observer coefficient of variation was 5.8% for the operator in the private and 4.1% for the one in the public sector, respectively, and the inter-observer coefficient of variation was 8.0% for measurements performed by the two operators. Both operators identified carotid artery bulb and/or internal carotid artery plaque in the same 11 of the 23 examined cases.

2.4. Altered plaque stability measures

Surrogate markers of plaque vulnerability levels comprising those of matrix metalloproteinase (MMP)-2 and -9 were quantified by a solid-phase sandwich ELISA (QuantikineHS). Their lower detection limits were 3.5 ng/ml, and 10 pg/ml, respectively; the interassay and intra-assay coefficients of variation were 10.0% and 12.0% for each of the 2 measurements.

2.5. Apelin concentrations

Concentrations of apelin C-terminal fragments that are responsible for the receptor binding and biological activity of apelin, were determined by a solid-phase sandwich ELISA

(QuantikineHS). The lower detection limit and inter- and intra-assay coefficients of variation were 0.1 ng/ml and <10% and <15%, respectively.

2.6. Data management and analysis

We expressed continuous variables as mean (SD) or median (interquartile range (IQR)) and applied logarithmic transformation as and when appropriate [28]. Categorical data are given as percentages.

Demographic characteristic-apelin level associations were assessed in a multivariable linear regression model. Other baseline characteristic apelin relations were determined in models that adjusted for demographic features. The independent apelin metabolic risk factor associations were assessed in linear or logistic regression models as appropriate and with adjustment for potential confounders as identified in the above mentioned multivariable regression analysis at $p < 0.2$.

The independent apelin -cIMT, -plaque and -altered plaque stability mediator levels were determined using linear or logistic regression models with adjustment for the Framingham score and additional potential confounders, as found in the foregoing analysis.

We have previously reported that the effects of adipokines are influenced by physiological status in RA [28,34,35]. For example, chemerin levels are associated with cIMT in overweight and obese but not normal weight RA patients [28]. Also, adiponectin concentrations relate independently to carotid plaque presence in RA patients with but not without abdominal obesity or no joint deformities [34]. In view of these findings, we determined the influence of patient characteristics on apelin-subclinical CVD relations by interaction analysis and performing stratified analysis when indicated (significant interaction p values) [28]. In this context, A BMI of ≥ 30 kg/m² and the presence of the metabolic syndrome (MetS) waist criterion [36] were considered to represent overall and abdominal obesity, respectively. When appropriate, subgroups were determined by median values.

We employed the IBM SPSS statistics program (version 22.0, IBM, USA). A $p \leq 0.05$ was considered significant.

3. Results

Descriptive statistics are given in Table 1. Women comprised 83.4% of the study population. The mean (SD) age and disease duration were 57.0 (10.8) and 13.6 (9.3) years, respectively. Hypertension, dyslipidemia and diabetes were recorded in 59.6, 17.9 and 12.3% of the participants, respectively, and 40.0% of them had carotid artery plaque. One or more synthetic disease modifying agents were used by each patient. The median (IQR) CDAI and mean (SD) DAS28 were 7 (2–14) and 2.9 (1.7) respectively, which reflect low disease activity [37]. The median (IQR) apelin concentrations were 390 (274–653) pg/ml.

3.1. Associations of baseline characteristics with apelin concentrations

Table 2 shows that black ethnicity was associated with low apelin concentrations; rheumatoid factor positivity and extra-articular manifestations related to high apelin levels. Other baseline characteristics that were related to apelin concentrations at $p > 0.05$ to <0.2 , and therefore included in subsequent multivariable regression models as potential confounders or/and mediators,

Table 1. Recorded characteristics in 235 patients with RA.

Demographic characteristics	
Age at study time, yrs	57.0 (10.8)
Female sex	83.4%
Black	48.5%
White	51.5%
Lifestyle factors	
Exercise	36.6%
Alcohol use	20.4%
Current smoking	6.4%
Anthropometry	
Body mass index, kg/m ²	27.5 (6.0)
Waist circumference, cm	91 (13)
Waist-hip ratio	0.86 (0.80–0.91)
Cardiovascular agents	
Antihypertensives	48.5%
Statins	28.1%
Ezetimibe	0.85%
Oral glucose-lowering agents	8.9%
Insulin	1.7%
RA characteristics	
Disease duration, yrs	13.6 (9.3)
Rheumatoid factor-positive	75.3%
Clinical Disease Activity Index	7 (2–14)
Disease Activity Score in 28 joints	2.9 (1.7)
Erythrocyte sedimentation rate, mm/h	12 (5–28)
C-reactive protein, mg/L	5.2 (2.2–12.3)
Interleukin 6, pg/ml	3.3 (2.2–6.0)
Leukocytes, n/ml	5.8 (4.8–7.6)
Deformed joints, no	6 (0–15)
Extra-articular manifestations	7.7%
Synthetic disease-modifying agents	
Methotrexate	84.3%
Chloroquine	66.4%
Leflunomide	29.8%
Sulfasalazine	18.7%
Azathioprine	15.0%
Tetracycline	11.9%
Cyclophosphamide	3.4%
Penicillamine	3.0%
Number	2.4 (0.9)
Prednisone use	2.1%
Tumor necrosis factor- α blockade	3.8%
CKD-EPI, ml/min/1.73 ²	95 (20)
Framingham score	2 (1–5)
Metabolic risk factors	
Conventional	
Hypertension	59.6%
Systolic blood pressure, mmHg	134 (22)
Diastolic blood pressure, mmHg	82 (13)
Total cholesterol, mmol/L	4.8 (1)
HDL cholesterol, mmol/L	1.54 (1.30–1.89)
LDL cholesterol, mmol/L	2.7 (0.8)
Triglycerides, mmol/L	1 (0.8–1.4)
Cholesterol-HDL cholesterol ratio	3.1 (2.5–3.6)
Cholesterol-HDL cholesterol ratio > 4	17.9%
Non-HDL cholesterol, mmol/L	3.2 (1)
Diabetes	12.3%
Glucose, mmol/L	4.7 (4.4–5.2)
Carotid atherosclerosis	
Intima-media thickness, mm	0.709 (0.110)
Plaque	40.0%
Plaque vulnerability	
MMP-2 (ng/ml)	1499 (604–2373)
MMP-9 (ng/ml)	4610 (3431–6842)
Apelin (pg/ml)	390 (274–653)

Dichotomous variables are expressed as proportions or percentages and continuous variables as mean (SD) or median (interquartile range).

RA, rheumatoid arthritis; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; HDL, high density lipoprotein; LDL, low density lipoprotein; MMP, matrix metalloproteinase.

are given in Table 2. Adiposity measures were not associated with apelin levels.

Table 2. Associations of demographic features, and other baseline characteristics with apelin. Concentrations, as well as of the latter with metabolic risk outcomes at $p < 0.2$.

Characteristics	β (SE)/OR (95% CI)	p value
Demographic characteristics		
Age at study time	0.000 (0.002)	0.9
Female sex	0.070 (0.048)	0.15
Black	−0.079 (0.036)	0.03
Alcohol use	0.085 (0.049)	0.08
RA characteristics		
Rheumatoid factor-positive	0.108 (0.041)	0.009
Erythrocyte sedimentation rate ^a	0.065 (0.043)	0.13
Extra articular manifestations	0.191 (0.067)	0.005
Synthetic disease-modifying agents		
Tetracycline use	0.075 (0.055)	0.18
Metabolic risk factors		
Systolic blood pressure	10.701 (5.183)	0.04
Diastolic blood pressure	7.236 (2.942)	0.02
Cholesterol/HDL cholesterol ratio >4	2.95 (0.73–11.97)	0.13

Baseline characteristic-apelin concentration relations were assessed in demographic variable adjusted models. Relations of apelin concentrations with metabolic risk factors were evaluated in demographic characteristics as well as alcohol use, rheumatoid factor status, erythrocyte sedimentation rate, extra-articular manifestations and tetracycline use adjusted models. Significant associations are shown in bold.

B, regression coefficient; SE, standard error; OR, odds ratio; CI, confidence interval; RA, rheumatoid arthritis; HDL, high-density lipoprotein.

^a Logarithmically transformed variables.

3.2. Independent relationships of apelin concentrations with metabolic risk

As also given in Table 2, systolic and diastolic blood pressure was significantly associated with apelin concentrations. After further adjustment for the use of antihypertensive drugs, the apelin-systolic blood pressure relation was no longer significant (β (SE) = 7.883 (4.820), $p = 0.1$) whereas the apelin-diastolic blood pressure association remained significant (β (SE) = 5.742 (2.754), $p = 0.03$). The p value for the cholesterol-HDL cholesterol ratio > 4–apelin relation was <0.2 (i.e. 0.13) but not significant. Upon additional adjustment for the use of lipid lowering agents, the respective p value was reduced to 0.08 with an odds ratio (95% confidence interval) of 3.16 (0.87–11.45). In subsequent multivariable analyses, non-modifiable and modifiable major conventional risk factors were accounted for by adjusting for the Framingham score [38] and to avoid overfitting.

3.3. Independent relations of apelin concentrations with atherosclerosis and altered plaque stability mediator levels

Table 3 shows that apelin concentrations were strongly related

to high MMP-2 and low MMP-9 levels in both univariate and adjusted analysis amongst all patients. Upon additional adjustment for BMI, CDAI and joint deformity counts, apelin concentrations remained associated with those of MMP-2 and MMP-9 (β (SE) = 0.355 (0.115), $p = 0.002$ and β (SE) = −0.243 (0.062), $p = 0.000$). Upon replacement of the CDAI by DAS28 in the latter model, apelin levels also remained related to those of MMP-2 and MMP-9 (β (SE) = 0.349 (0.115), $p = 0.000$ and β (SE) = −0.247 (0.115), $p = 0.003$). Apelin concentrations were not related to cIMT and plaque in all patients.

3.4. Impact of patient characteristics on the associations of apelin concentrations with carotid atherosclerosis and plaque stability mediator levels

The DAS28 and CDAI impacted on the apelin concentration-cIMT relations (interaction $p = 0.04$ and 0.001, respectively), the ESR impacted on the apelin concentration-MMP-2 level relations (interaction $p = 0.04$) and population origin and IL-6 levels impacted on apelin concentration-MMP-9 relations (interaction $p = 0.03$ and $p = 0.04$, respectively).

As given in Table 4, in stratified analysis, apelin concentrations were inversely associated with cIMT in patients with RA remission or low but not moderate or high disease activity. Apelin levels were directly related to those of MMP-2 in white but not black patients and only in those without (but not with) large erythrocyte sedimentation rates or interleukin-6 values or moderate or high disease activity as assessed by DAS28. Apelin concentrations were associated with those of MMP-2 irrespective of disease activity status as evaluated by CDAI. The apelin-MMP-9 relation was reproduced in all subgroups of interest except for those with high disease activity, as assessed by DAS28, in whom it was not significant ($p = 0.2$). However, the apelin-MMP-9 association did not differ in patients with versus those without a DAS28 > 3.6 (interaction $p = 0.7$).

4. Discussion

The present study demonstrates that apelin concentrations are strongly and inversely associated with those of MMP-9 in patients with RA. In stratified analysis, these findings were consistently reproduced amongst both black and white patients and those with and without a large inflammatory burden. MMP expression is altered in obesity [39] and can represent disease activity [40] and joint damage [41] in RA. The apelin-MMP relations in this investigation were independent of adiposity measures, the CDAI or DAS28 and deformity joint count as well as a range of other potential confounders. Apelin concentrations were also associated with those of MMP-9 irrespective of RA activity as evaluated by CDAI, but not significantly so in patients with moderate or high disease

Table 3. Relationship of apelin concentrations with carotid atherosclerosis and levels of altered plaque stability mediators.

Characteristics	Univariate		Multivariate	
	β (SE)	p	β (SE)	p
Carotid atherosclerosis				
Intima-media thickness	−0.231 (0.157)	0.2	0.041 (0.026)	0.1
	OR (95% CI)	p	OR (95% CI)	p
Plaque	1.052 (0.401–2.684)	0.9	0.763 (0.524–2.313)	0.6
Plaque vulnerability	B (SE)	p	β (SE)	p
MMP-2 ^a	0.103 (0.037)	0.01	0.324 (0.112)	0.004
MMP-9 ^a	−0.321 (0.069)	0.000	−0.239 (0.060)	0.000

Relations were assessed in race, Framingham score, rheumatoid factor status, erythrocyte sedimentation rate, extra-articular manifestations, tetracycline use and alcohol use adjusted multivariable models. Significant associations are shown in bold.

B, regression coefficient; SE, standard error; MMP, matrix metalloproteinase.

^a Logarithmically transformed variables.

Table 4. Independent relationships of apelin concentrations with carotid intima-media thickness and levels of altered plaque stability mediators in subgroups.

Groups	Number	Relationship with atherosclerosis and altered plaque stability mediator levels					
		cIMT		MMP-2		MMP-9	
		β (SE)	<i>p</i> value	β (SE)	<i>p</i> value	β (SE)	<i>p</i> value
Population							
White	121	−0.018 (0.037)	0.6	0.367 (0.146)	0.01	−0.192 (0.072)	0.01
Black	114	−0.012 (0.044)	0.8	0.197 (0.220)	0.4	−0.269 (0.128)	0.04
Missing	0						
CDAI >10							
No	140	−0.068 (0.033)	0.04	0.304 (0.150)	0.04	−0.239 (0.075)	0.002
Yes	94	0.015 (0.046)	0.7	0.354 (0.177)	0.05	−0.207 (0.104)	0.05
Missing	1						
DAS28 > 3.6							
No	165	−0.062 (0.031)	0.04	0.343 (0.140)	0.01	−0.242 (0.066)	0.000
Yes	70	0.006 (0.057)	0.9	0.233 (0.209)	0.2	−0.189 (0.149)	0.2
Missing	1						
ESR >12 mm/h							
No	119	−0.035 (0.038)	0.4	0.428 (0.143)	0.003	−0.227 (0.073)	0.003
Yes	110	−0.030 (0.035)	0.4	0.115 (0.199)	0.6	−0.279 (0.112)	0.02
Missing	6						
IL-6 > 3.25 pg/ml							
No	106	−0.063 (0.046)	0.2	0.485 (0.288)	0.04	−0.225 (0.095)	0.02
Yes	110	−0.013 (0.035)	0.7	0.254 (0.140)	0.08	−0.335 (0.093)	0.000
Missing	19						

Relations were assessed in race, Framingham score, rheumatoid factor status, erythrocyte sedimentation rate, extra-articular manifestations, tetracycline and alcohol use adjusted multivariable models. Significant associations are shown in bold.

cIMT, carotid intima-media thickness; MMP, matrix metalloproteinase; β , regression coefficient; SE, standard error.

activity as assessed by DAS28. The apelin-MMP-9 did not differ amongst patients with and without a high DAS28 as the related interaction *p* was 0.7. Moreover, acute phase responses are reportedly not associated with CVD amongst African black RA patients [42] and are included in the DAS28. Hence, the CDAI is likely to be a more accurate measure of disease activity than the DAS28 in the present context.

Apelin was shown to downregulate the production of MMP-9 [43]. MMP-9 expression is increased in human foam cell macrophages and causes extracellular matrix destruction [17]. Circulating concentrations of MMP-9 are increased in patients with acute coronary artery syndrome [18]. An MMP-9 polymorphism that increases its transcription and CAD was reported [17]. These findings together with the current results suggest that apelin may reduce plaque vulnerability in RA.

Apelin concentrations were directly related to those of MMP-2 in the present investigation. Apelin was previously reported to increase the expression of MMP-2 [44]. Although MMP-2 concentrations are also increased after a myocardial infarction, this is thought to represent a consequence rather than a cause [17]. Indeed, in contrast to MMP-9, MMP-2 mediates plaque stability [17,18].

Importantly in the context of RA, we found that systemic inflammation modified the apelin-MMP-2 relation. Accordingly, the potential enhancing effect of apelin on plaque stability was absent in patients with an ESR or IL-6 concentration above their median value. This abrogated effect of apelin may, therefore, contribute to the reported increased CVD risk associated with systemic inflammation in RA [19]. Our findings have implications in CVD risk management through adequate inflammation control.

Markers of systemic inflammation including the ESR, CRP and IL-6 concentrations as well as disease activity measures comprising the CDAI and DAS28 were not associated with apelin concentrations in this study. Also, amongst all RA patients, apelin levels were not related to atherosclerosis measures. However, apelin concentrations were inversely associated with cIMT in patients with disease remission or mild but not moderate or high disease activity as assessed by CDAI or DAS28. This may suggest a disassociation

between inflammation and vascular disease in RA patients with low or absent disease activity. As apelin concentrations were decreased in patients with untreated early RA [25], adequate RA activity control, as was observed in the majority of patients in the present investigation, may increase apelin levels and thereby reduce CVD risk. These possibilities should be studied in future longitudinal investigations. The present findings on the apelin-cIMT association may reinforce the need for tight disease activity control in CVD prevention amongst patients with RA.

In this study, apelin concentrations were lower in black compared to white RA patients. Further, in contrast to their white counterparts, black patients did not experience a direct apelin-MMP-2 relation. Different associations of polymorphisms in adipokine related genes with subclinical CVD amongst black and white participants were previously reported in the multi-ethnic study of atherosclerosis [45]. Population specific CVD risk stratification is indicated amongst patients with RA [46].

Our analysis revealed a paradoxically direct association between apelin concentrations and systolic and diastolic blood pressure. Cardiovascular drugs can impact disease activity and traditional and non-traditional cardiovascular risk factors in RA [47]. Indeed, upon additional adjustment for antihypertensive agent use, the apelin level-systolic blood pressure relation was no longer significant. Nevertheless, the apelin concentration-diastolic blood pressure association was independent of antihypertensive agent use. In this regard, it is noteworthy that adiponectin, which is another vascular protective molecule, reportedly shows similar relationships to blood pressure values in RA [48]. The vasorelaxing effects of apelin are endothelium dependent [49] and RA patients experience endothelial activation [50] and dysfunction [51]. Nevertheless, the respective paradoxical relationships likely represent a compensatory process in the presence of heightened CVD risk and aimed at reducing this risk [48].

Whereas smoking is an important cardiovascular risk factor and enhances RA development and severity, only 6.4% of participants in the present study were current smokers. This was at least partly due to the inclusion of black African patients that are at an earlier epidemiological health transition stage [46]. Overall, 10% of white

and 4% of black RA patients were current smokers. Also, 48% of white and 12% of black patients were ever smokers.

Only 3.8% of RA patients in this investigation employed anti-TNF- α agents despite a mean disease duration of 13.6 years. This is because at the time of the study, patients seen at the Charlotte Maxeke Johannesburg Academic Hospital (public healthcare) had no access to biological therapy while the use of the respective intervention was also restricted in the Milpark hospital (private healthcare).

The Framingham score was non-normally distributed in the current investigation with a median value 2% (interquartile range = 1%–5%). The mean (SD) Framingham score was 5.3 (6.7%).

The current investigation comprises a relatively large group of comprehensively evaluated RA patients from two population groups. We systematically explored potential confounders. Nevertheless, other as yet unidentified confounding factors may exist as RA is a complex disease. Moreover, many novel biomarkers of CVD risk have pleiotropic effects that may vary over time and at different disease stages of RA. In addition, our study design was cross-sectional, which precludes drawing inferences on the direction of causality. We assessed carotid IMT and plaque as markers of pre-clinical CVD. Other validated methods for the assessment of sub-clinical atherosclerosis in rheumatic diseases comprise the evaluation of endothelial dysfunction by brachial artery flow mediated dilatation (FMD) and arterial stiffness by pulse wave velocity (PWV) [52]. Low vitamin D and apo A-1 were recently found to be associated with increased PWV [53,54] in RA. Future studies assessing the potential influence of apelin on FMD and PWV may further elucidate the role of this adipokine in CVD amongst RA patients.

In conclusion, this study documents for the first time favorable independent relationships between apelin concentrations and altered plaque stability mediator levels and atherosclerosis in RA. These associations are partially dependent on population origin and systemic inflammation status. The potential role of apelin in CVD risk and its stratification merit further investigation in future longitudinal studies.

Conflict of interest

The authors declare that they do not have anything to disclose regarding conflict of interest with respect to the manuscript.

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Author contributions

PHD, AS and LT designed the study and performed the data collection. AS performed the carotid ultrasound examinations in patients at the Charlotte Maxeke Johannesburg Academic Hospital. PHD, SG and LT performed the data analysis. PHD and SG wrote the manuscript. All authors contributed to the interpretation of the results and approved the final version of the manuscript.

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Paper 4 - Apelin concentrations are associated with altered atherosclerotic plaque stability mediator levels and atherosclerosis in rheumatoid arthritis

In paper 4, apelin concentrations were favorably associated to those of MMP-9, a plaque vulnerability marker, and MMP-2, a plaque stability marker. In line with our findings in paper 3, systemic inflammation impacted the apelin-MMP-2 relationship.

Summary and conclusions

This thesis comprises 4 studies investigating factors related to arterial function and atherosclerosis in RA. Patients were recruited at the Charlotte Maxeke Johannesburg Academic Hospital (paper 4) and Milpark Hospital in Johannesburg, South Africa.

Arterial stiffness reportedly predicts cardiovascular events in both non-RA and RA populations, independent of traditional cardiovascular risk factors (Klocke *et al.*, 2003; Mattace-Raso *et al.*, 2006; Mitchell *et al.*, 2010). Arterial stiffness as measured by PWV and wave reflection as estimated by Alx comprise the two arterial function measures that were previously most frequently investigated in studies on CVD risk stratification (Mattace-Raso *et al.*, 2006; Laurent *et al.*, 2006; Vlachopoulos *et al.*, 2010^a; Ambrosino *et al.*, 2015). We additionally included the measurement of wave reflection and pressure pulsatility, as these factors were also shown to independently contribute to CVD risk in non-RA persons (Safar *et al.*, 2003; Mitchell *et al.*, 2004; Weber *et al.*, 2004; Wang *et al.*, 2010).

In the present study, we identified several traditional and novel cardiovascular risk factors as well as RA characteristics that were significantly associated with measures of arterial function, independent of established confounders, which comprise age, blood pressure, height and heart. Notably, several disease specific markers were associated with wave reflection and pressure pulsatility, but not with PWV or Alx. For example, RA duration and rheumatoid factor positivity, which represent markers of cumulative inflammation or disease severity, were independently associated with central pulse pressure and wave reflection measures, but were not related to PWV or Alx. Rheumatoid factor status was further associated with reduced pulse pressure amplification. The leukocyte count was associated with central pulse pressure and wave reflection, but related to neither PWV nor Alx.

Overall, patient characteristics explained 37%, 71% and 68% of the PWV, wave reflection and pressure pulsatility variability, respectively. RA characteristics were more strongly and consistently

related to wave reflection and pressure pulsatility than arterial stiffness. These findings support the consideration of comprehensive arterial function evaluation in RA. A further important clinical implication of our findings is that through disease activity control, treatment of cardiovascular risk factors and life style intervention, arterial function may be preserved in RA patients. The 2015/2016 EULAR recommendations for prevention of CVD in inflammatory arthritis emphasized not only disease control and cardiovascular risk factor management but also life style intervention, including a healthy diet, regular exercise and smoking cessation (Agca *et al.*, 2017). Our results reinforce the importance of the respective recommendations.

Impaired arterial function reportedly causes heart disease by increasing cardiac workload and reducing coronary perfusion in non-RA persons (Vlachopoulos *et al.*, 2010^b; Townsend *et al.*, 2015). Arterial stiffening enhances wave reflection, whereby each of these properties simultaneously promote broadening of pulse pressure (Townsend *et al.*, 2015). Increased pulse pressure may promote atherosclerosis (Dart *et al.*, 2001). Patients with RA are particularly prone to atherosclerotic CVD (Agca *et al.*, 2017; Avina-Zubeita *et al.*, 2008). In this regard, arterial wave reflection occurs at arterial branching points, where perturbed blood flow increases the risk of atherosclerosis. Thus, the hemodynamic changes associated with arterial dysfunction are also related to the development of atherosclerosis (Laurent *et al.*, 2006; Townsend *et al.*, 2015).

Hence, in this second study, we set out to determine whether and which arterial function measures are associated with atherosclerosis in patients with RA. We wished to determine especially whether arterial wave reflection, as quantified in wave separation analysis, is related to markers of atherosclerosis.

We indeed demonstrated that wave reflection measures including reflected wave pressure and reflection magnitude, are strongly associated with the presence of ultrasound determined carotid plaque. Although traditional cardiovascular risk factors impact arterial function as was reported in paper 1, the arterial function-atherosclerosis relations identified in paper 2 were independent of traditional cardiovascular risk factors. Further, the arterial function-atherosclerosis relations were also independent of arterial stiffness as estimated by PWV. While emphasis is often placed on

adequate and timely traditional risk factor management, it appears pertinent in the present context that arterial dysfunction in the RA population can occur independent of traditional cardiovascular risk factors (Klocke *et al.*, 2003; Van Doornum *et al.*, 2003). Moreover, upon receiver operator characteristic curve analysis, we found that at a cut-off value of 25 mmHg, reflected wave pressure was sensitive and specific to a clinically useful extent in predicting plaque presence. We concluded that arterial wave reflection evaluation may improve CVD risk stratification beyond brachial blood pressure and conventional risk scores in patients with RA. Further, specific targeting of increased wave reflection, for example by using angiotensin converting enzyme inhibitors, angiotensin receptor blockers or calcium channel blockers rather than beta blockers as first line antihypertensive agents, may reduce CVD risk in RA.

Whereas traditional cardiovascular risk factor exposure and systemic inflammation may promote arterial dysfunction and enhance atherogenesis in RA, the role of more novel cardiovascular risk markers in arteriosclerosis and atherosclerosis remains uncertain in this disease. In this regard, apelin increases both cardiac contractility and vasodilation (Yamaleyeva *et al.*, 2016). The vasoactive action of apelin results in improved arterial function amongst patients with an intact endothelium (Japp *et al.*, 2008^a; Han *et al.*, 2013). Notably, systemic inflammation is associated with endothelial dysfunction in patients with RA. Conceptually, this may therefore result in a diminished protective effect of apelin on the vasculature.

In our third study, we investigated whether apelin concentrations were associated with arterial function. Although apelin levels were not associated with arterial stiffness, wave reflection or pressure pulsatility in all patients, we demonstrated that the inflammatory burden markedly impacted on apelin concentration-pressure pulsatility relations. We therefore concluded that apelin may protect against adverse proximal aortic modeling and impaired function in RA. However, a concurrent increased current or cumulative inflammatory burden may abrogate this vascular protective effect of apelin in RA. In the absence of the respective effect installed by apelin, an enhanced risk of CVD would be expected, particularly among patients with active RA.

Adequate systemic inflammation control may therefore not only reduce RA activity and physical function but also enhance apelin-mediated protection against detrimental proximal aortic remodeling. Potential adverse effects of systemic inflammation were also noted in paper 1 where inflammation markers were associated with measures of arterial function including arterial stiffness, wave reflection as well as pressure pulsatility.

The results of the third paper also substantiate our conclusions of the first 2 manuscripts of the present thesis in that apelin concentrations were associated with pressure pulsatility but not arterial stiffness. Indeed, this again reinforces the potential role of thorough arterial function assessment in RA patients as determinants of the various aspects of arterial function are highly variable.

Our findings in paper 3 raise the question as to whether apelin may protect against CVD mediated by atherosclerosis and whether systemic inflammation influences this process. In the final investigation of this study, we assessed potential anti-atherogenic properties of apelin in patients with RA by determining whether its concentrations are associated with markers of atherosclerosis. We found that apelin levels were inversely associated with those of MMP-9, a marker of plaque vulnerability, in RA. By contrast, in line with our findings on apelin-arterial function associations in paper 3, systemic inflammation markedly modified apelin-MMP-2 relations in RA. Apelin was favorably associated with MMP-2, a plaque stability marker, in RA patients without but not with a large inflammatory burden.

In summary, in the context of the reportedly high prevalence of atherosclerosis in RA, our current results indicate that apelin may play an important role in plaque stabilization among patients with this disease. Further, our results suggest that reduced apelin effects may mediate the reported link between disease activity and increased CVD risk in RA. Adequate RA activity and inflammation control may be pivotal in preserving the cardioprotective effects exerted by apelin. Finally, apelin may have potential as a therapeutic target in attempts at improving arterial function, reducing atherosclerotic risk and promoting plaque stability amongst patients with RA.

Taken together, the main findings of this thesis are that: (1) disease characteristics and traditional cardiovascular risk factors are independently associated with comprehensively assessed arterial function; (2) large artery wave reflection, as determined in separation analysis, and pressure pulsatility are related to the presence of advanced atherosclerosis independent of other CVD risk factors and (3) apelin may improve arterial function and promote plaque stability particularly in the absence but not presence of increased systemic inflammation and/or disease activity in patients with RA. On the whole, our results further support the observation that the management of not only traditional cardiovascular risk factors but also disease activity and inflammation is paramount in reducing the overall CVD risk in RA. Finally, the consideration and use of comprehensive arterial function evaluation may improve CVD risk stratification in patients with RA.

The studies that were included in this thesis have strengths and limitations. We evaluated a comprehensive set of arterial function measures in a relatively large multi-ethnic population group. We systematically considered recognized confounders and explored additional potential ones. Nevertheless, given the complexity of RA, other unidentified confounders may still exist. We assessed a large number of associations and made use of backward regression models on cross sectional data. The main limitation of this study is its cross sectional design, whereby age, sex and ethnicity matched controls were not included. This precludes drawing conclusions on the direction of cause effect relationships. Further, associations may represent reciprocally reinforcing effects in the present context (Dart *et al.*, 2001; Laurent *et al.*, 2009; Laurent *et al.*, 2006; Safar *et al.*, 2003). Since we did not include a control group, it remains uncertain as to whether our findings are RA specific. We cannot clearly indicate whether these findings are in fact unique to RA and therefore, we cannot be certain if results are not merely due to the effects of traditional cardiovascular risk factors, especially aging. We recommend the inclusion of matched control groups in future longitudinal and interventional studies, in order to more accurately establish the effect that RA disease characteristics have on arterial function, atherosclerosis and subclinical CVD over and above the effects of traditional cardiovascular risk factors.

We measured arterial function using applanation tonometry and made use of a generalized transfer function to determine the aortic pressure waveform non-invasively. Although the use of generalized transfer functions are validated and have been used with success (Chen *et al.*, 1997; Fetis *et al.*, 1999), it does have limitations. The exact estimation of the aortic pressure waveform from the measurement of the brachial waveform is impeded by various confounding factors, including variable cushioning of the arteries, as well as modifications by reflections from the distal arterial bed (Hoeks *et al.*, 2003). Transfer functions have been shown to be less accurate in clinical populations such as diabetes mellitus II, in which there is a trend toward a greater Alx (Hope *et al.*, 2004^b). There is also some criticism regarding the calibration of central blood pressure. In this regard, it was shown that the central blood pressure is significantly underestimated when calibrating the central blood pressure by using non-invasive methods (Hope *et al.*, 2004^a; O'Rourke *et al.*, 2003).

Also, the use of a triangular waveform for the execution of wave separation analysis has been validated. Accordingly, the reflected wave pressure was shown to predict 15-year all cause cardiovascular mortality (Wang *et al.*, 2010). However, the use of triangular flow approximation may not be the best estimate for aortic flow (Kips *et al.*, 2009), as backward and forward wave pressures tend to be overestimated when compared to Doppler measurements (Kips *et al.*, 2009).

Finally, the additional evaluation of endothelial dysfunction as measured by flow mediated dilatation could have provided further evidence in support of our findings in the present study, particularly as relates to papers 3 and 4. This should be addressed in future studies.

Conclusion

In conclusion, traditional cardiovascular risk factors and disease characteristics are associated with core aspects of arterial function including arterial stiffness, wave reflection and pressure pulsatility in RA. Amongst these measures, increased arterial wave reflection was most strongly related to atherosclerosis in the present RA investigation. These findings call for comprehensive arterial function evaluation in RA. High apelin concentrations are associated with favorable plaque vulnerability marker concentrations as well as low pressure pulsatility in RA but these potential

protective effects may be partially abrogated in the presence of a large inflammatory burden. The potential role of arterial function and apelin in cardiovascular risk and its stratification among patients with RA merits further study. The findings in this thesis further support the reported recommendations to target both traditional cardiovascular risk management and adequate RA control in the prevention of CVD.

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Confirmation of contribution to the work by Supervisors

To Whom It May Concern,

We hereby confirm that Ms Sulé Gunter has substantially contributed to the conception of each of the studies, data collection, interpretation of the results and writing of the manuscripts that are included in this PhD thesis.

Yours sincerely,

A handwritten signature in black ink, appearing to be 'P. H. Dessen', written in a cursive style.

Prof Patrick H Dessen MD, FCP(SA),FRCP(UK), PhD

A handwritten signature in black ink, appearing to be 'Aletta ME Millen', written in a cursive style and enclosed within a circular flourish.

Associate Prof Aletta ME Millen, PhD



R14/49 Ms Sulé Gunter

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M150324

NAME: Ms Sulé Gunter

(Principal Investigator)

DEPARTMENT: Physiology
Milpark Hospital

PROJECT TITLE: Risk Factors for Subclinical Cardiovascular Disease
in Patients with Osteo and Rheumatoid Arthritis

DATE CONSIDERED: 27/03/2015

DECISION: Approved unconditionally

CONDITIONS: Title Change(11/08/2017)

SUPERVISOR: Dr A Millen and Prof P Dessein

APPROVED BY:



Professor P. Cleaton-Jones, Chairperson, HREC (Medical)

DATE OF APPROVAL: 29/05/2015

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary 3rd floor, Phillip Tobias Building, Parktown, University of the Witwatersrand. I/We fully understand the conditions under which I am/we are authorised to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit to the Committee. **I agree to submit a yearly progress report.** The date for annual re-certification will be one year after the date of convened meeting where the study was initially reviewed. In this case, the study was initially reviewed in March and will therefore be due in the month of March each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).

Principal Investigator Signature _____

Date _____

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES



UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG
Division of the Deputy Registrar (Research)

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
R14/49 Dr Patrick H Dessein

CLEARANCE CERTIFICATE

M120562

PROJECT

The Contribution of the Interaction of
Rheumatoid Arthritis Disease Activity with
Metabolic Syndrome Features to Endothelial
Dysfunction, Arterial Stiffness and Athero-
Sclerosis

INVESTIGATORS

Dr Patrick H Dessein.

DEPARTMENT

Department of Medicine

DATE CONSIDERED

Ad hoc

+DECISION OF THE COMMITTEE*

Renewal Approved

Unless otherwise specified this ethical clearance is valid for 5 years and may be renewed upon application.

DATE 31/05/2012

CHAIRPERSON


(Professor PE Cleaton-Jones)

*Guidelines for written 'informed consent' attached where applicable

cc: Supervisor : Prof B Joffe

DECLARATION OF INVESTIGATOR(S)

To be completed in duplicate and **ONE COPY** returned to the Secretary at Room 10004, 10th Floor, Senate House, University.

I/We fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be contemplated from the research procedure as approved I/we undertake to resubmit the protocol to the Committee. **I agree to a completion of a yearly progress report.**

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES...



R14/49 Prof Patrick Dessein et al

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
CLEARANCE CERTIFICATE NO. M170592

NAME: Prof Patrick Dessein et al
(Principal Investigator)
DEPARTMENT: School of Physiology
Internal Medicine

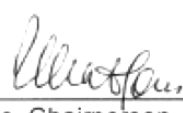
PROJECT TITLE: The Contribution of Interaction of Rheumatoid Arthritis
Diseas Activity with Metabolic Syndrome Features
to Endothelial Dysfunction, Arterial Stiffness and Athero-Sclerosis

DATE CONSIDERED: 31/05/2012 (Initial approval) 26/05/2017 (Recertified)

DECISION: Approved unconditionally

CONDITIONS: Renewal for the period May 2017 - May 2022
Previously M120562

SUPERVISOR: Prof B Joffe

APPROVED BY: 
Prof P Cleaton-Jones, Chairperson, HREC (Medical)

DATE OF APPROVAL: 12/06/2017

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary in Room 301, Third floor, Faculty of Health Sciences, Phillip Tobias Building, 29 Princess of Wales Terrace, Parktown, 2193, University of the Witwatersrand. I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. **I agree to submit a yearly progress report.** The date for annual re-certification will be one year after the date of convened meeting where the study was initially reviewed. In this case, the study was initially reviewed in May and will therefore be due in the month of May each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).

Principal Investigator Signature _____

Date _____

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES