OMENTIN AND SUBCLINICAL CARDIOVASCULAR DISEASE IN RHEUMATOID ARTHRITIS

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A dissertation submitted to the Faculty of Health Sciences, University of the Witwatersrand, in fulfilment of the requirements for the degree of Masters of Science in Medicine.

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DECLARATION

I, Chanel Robinson, declare that this dissertation is my own work. It is being submitted for the degree of Master of Science in Medicine in the Faculty of Health Sciences at the University of the Witwatersrand, Johannesburg, South Africa. I declare that the dissertation submitted is entirely my own, original work and the copy right of this dissertation rests with the author or the University to which it was submitted. The work herein has not been submitted previously for any degree or examination at this or any other University.

Chanel Robinson

Signed on the 22 nd day of June 2016

ABSTRACT

The enhanced cardiovascular disease (CVD) risk experienced by patients with rheumatoid arthritis (RA) remains largely unexplained. Traditional risk factors including hypertension, dyslipidemia, diabetes, smoking and altered adiposity states do not fully account for the increased CVD risk in RA. High grade systemic inflammation, as characteristically present in patients with RA, is associated with adverse metabolic risk factor profiles and can directly increase atherogenesis. Further, genetic polymorphisms are related to CVD in RA. Notably, the impact of cardiovascular risk factors on CVD is epidemiological health transition stage dependent within populations. Indeed, cardiovascular risk factor-CVD relations consistently differ amongst patients with RA from developed compared to developing populations. In line with these findings, adequate CVD risk stratification in RA currently eludes us.

Adipose tissue derived adipokines are major determinants of systemic metabolism. Several recent studies revealed that adipokines are involved in RA activity and severity. Adipokines play key roles in interactions between obesity, metabolic cardiovascular risk factors and systemic inflammation, all of which contribute to cardiovascular pathology. These adipokine effects depend on pathophysiological context. Against this background, in the present study, we investigated the associations of omentin concentrations with subclinical CVD and whether population origin and RA activity and severity impacts on the respective relationships.

Omentin concentrations were measured in 213 (104 black; 109 white) RA patients. Relationships of omentin levels with those of 6 endothelial activation markers, ultrasound determined carotid intima-media thickness and plaque, and matrix

metalloproteinase (MMP)-2, -3 and -9 that mediate plaque stability, were identified in multivariate regression models.

Omentin concentrations were inversely associated with MMP-3 levels (β=-364 (0.113), p=0.002). This relationship was influenced by population origin, RA activity and the erythrocyte sedimentation rate (ESR) and joint deformity count (interaction p value=0.009, 0.04, 0.04 and 0.007, respectively). Accordingly, in stratified analysis, the omentin-MMP-3 concentration relationship was reproduced in white (β (SE)=-0.450 (0.153), p=0.0004) but not black patients (β (SE)=- 0.099 (0.195), p=0.6), in participants with disease remission or mild disease activity (β (SE)=-0.411 (0.139), p=0.004) but not with moderate or severe RA activity (β (SE)=-0.286 (0.202), p=0.2), and in those with a small (β (SE)=-0.534 (0.161), p=0.001) but not large erythrocyte sedimentation rate (ESR) (-0.212 (0.168), p=0.2) and without (β (SE)=-0.554 (0.165), p=0.0001) but not with large joint deformity counts (-0.110 (0.173), p=0.5). Omentin levels were unrelated to endothelial activation and atherosclerosis.

Omentin concentrations do not represent endothelial activation and atherosclerosis extent in RA. However, omentin concentrations were inversely associated with those of MMP-3, a surrogate marker of plaque vulnerability to rupture, in white but not black Africans with RA. This inverse relationship was also absent RA patients with moderate or severe RA activity and large ESR values and joint deformity counts. A loss of beneficial effects of omentin on plaque instability may contribute to the link between severe disease and increased cardiovascular risk in RA.

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ACPA	anti-citrullinated protein
ADMA	asymmetrical dimethylarginine
CDAI	clinical disease activity index
CKD-EPI	chronic kidney disease epidemiology collaboration
CV	cardiovascular
CVD	cardiovascular disease
EFGR	estimated glomerular filtration rate
HDL	high density lipoprotein
ICAM	intercellular adhesion molecule
IL-6	interleukin 6
MCP	monocyte chemoattractant protein
RA	rheumatoid arthritis
RBP-4	retinol binding protein-4
SVF	stromal vascular fraction
TNF	tumour necrosis factor
VCAM	vascular adhesion molecule

CHAPTER 1

INTRODUCTION

1.1 RHEUMATOID ARTHRITIS

Rheumatoid arthritis (RA) is a prototypic chronic inflammatory condition that affects 0.5 to 1 % of the population. The core characteristic of RA is synovitis that results in the destruction of cartilage and bone. In addition to synovial inflammation and hyperplasia, rheumatoid arthritis is characterised by autoantibody production (anti-citrullinated protein antibody [ACPA]) and rheumatoid factor) with systemic inflammation resulting in additional complications compromising multiple organ systems (McInnes & Schett, 2011). In the presence of extraarticular manifestations and comorbidities, this autoimmune disease is characterised by substantial disability as well as an increase in patient mortality (Alamanos & Drosos, 2004).

RA comprise several disease subsets with disease manifestations that involve disparate inflammatory cascades (Scott e*t al.*, 2010; Van der Helm-van Mil & Huizinga, 2008). One such cascade comprises the overproduction and overexpression of tumor necrosis factor (TNF) (Feldman e*t al.*, 1996; Scott e*t al.*, 2010) eliciting a response from additional cytokines such as interleukin-6 (IL-6). Overproduction of inflammatory cytokines is stimulated by abnormal activity of synovial cells or synoviocytes within the joints of RA patients. A role of osteoclast activation in joint destruction has also been suggested (Cohen e*t al.*, 2008).

Although the exact etiology is unknown, RA comprises complex interactions between genetic- and environmental factors (Scott *et al.*, 2010; McInnes & Schett, 2011; Gibofsky *et al.*, 1978). Twin studies yielding concordance rates of 15-30 % in monozygotic twins and 5 % in dizygotic twins (Silman & Pearson, 2002), suggest that RA prevalence can be attributed to genetic factors (McInnes & Schett, 2011;

Gibofsky 2012). In addition to twin studies, the shared epitope hypothesis suggests the impact of class II molecules in the pathogenesis of rheumatoid arthritis. The human leukocyte antigen (HLA)-DRB1 locus confers disease susceptibility in patients who test positive for rheumatoid factor and/or ACPA (Gregersen *et al.*, 1987; Mc Innes & Schett, 2011; Gibofsky 2012). Gene interactions between (HLA)-DRB1 and PTPN22 have been described thereby adding to the complexity of the exact etiology in rheumatoid patients (Karlberg *et al.*, 2007). With regards to gene-environment interactions, smoking confers increased risk for rheumatoid arthritis in patients presenting (HLA)-DR4 alleles (Symmons *et al.*, 1997). Synergistically, smoking confers increased risk for ACPA-positive disease in the presence of (HLA)-DRB1 alleles (McInnes & Schett, 2011; Klareskog *et al.*, 2006).

Infectious agents triggering the initiation and formation of immune complexes have also been shown to associate with RA (Auger & Roudier, 1997; Kamphuis *et al.*, 2005). Infectious agents implicated in the pathogenesis of rheumatoid arthritis include Epstein-Barr virus, cytomegalovirus, parvo- and rubella virus (Alamanos & Drosos, 2004).

In addition to the above, most RA patients are women. Studies suggest an increased incidence of rheumatoid arthritis in women with their susceptibility being increased in the presence of oral contraceptives and reduced with parity (Silman & Pearson 2002, Alamanos & Drosos, 2004, Gibofsky, 2012).

1.2 CARDIOVASCULAR RISK IN RHEUMATOID ARTHRITIS

Where cardiovascular disease (CVD) accounted for less than 10 % of global mortality at the beginning of the 20th century, it is now estimated that 30 % of global deaths are attributable to diseases of the cardiovascular system (Gaziano, 2005). Almost 80 % of the CVD burden occurs in developing countries (Yusuf e*t al.*, 2004).

It is well established that RA confers increased risk for cardiovascular disease including ischemic heart disease and cerebrovascular accidents (Avina-Zubieta *et al.*, 2008; Gonzalez-Gay *et al.*, 2005; Dessein *et al.*, 2007; Gonzalez-Gay *et al.*, 2007; Rodriguez-Rodriguez *et al.*, 2012). A meta-analysis by Avina-Zubieta et al (2008) concludes that the CVD mortality rate is increased by as much as 50 % in RA patients as compared to that in the general population.

Genetic determinants contribute to the increased risk of CVD and mortality experienced by RA (Gonzalez-Gay et al, 2007). Additionally, high grade inflammation and the impact of inflammatory changes on traditional risk factors is most pertinent in the present context (Dessein *et al.*, 2007). The development of accelerated atherosclerosis in response to chronic inflammatory responses contributes to the increased CVD risk observed in patients with rheumatoid arthritis (Gonzalez-Gay *et al.*, 2005; Gonzalez-Gay *et al.*, *2007*). Although traditional risk factors including dyslipidaemia, hypertension, smoking and diabetes have repeatedly been shown to predict cardiovascular events in a general population (Menotti *et al*., 2009; Yusuf *et al.*, 2004, Yusuf *et al*., 2001b; O'Donnell *et al*., 2010), these conventional risk factors do not fully account for the adverse outcomes in patients with RA (del Rincon, 2001). In this regard, alternative CVD risk profiling strategies need to be evaluated in specific RA populations. This requires consideration of

differences in epidemiological disease health transition stages amongst persons living in developed countries and developing countries, an issue that is particularly relevant to African populations.

Solomon *et al.* recently reviewed findings on cardiovascular risk factors and subclinical CVD in black African patients with RA from a developing population. Despite marked RA activity and increased severity observed in this group (Solomon e*t al.*, 2005) less frequent atherosclerotic CVD has been reported in this population at large. Dessein *et al.* (2013e) investigated cardiovascular risk factor profiles and their associations with carotid atherosclerotic changes in black Africans with RA. Despite a higher prevalence of hypertension and diabetes, both conventional and non-conventional CVD risk burdens as well as arterial stiffness were found to be similar in black compared to white Africans (Solomon *et al.*, 2010; Dessein *et al.*, 2013b). Unexpectedly, a similar atherosclerosis burden was noted in black compared to white African RA patients (Solomon *et al.*, 2012). Traditional and non-traditional risk factors were independently related to atherosclerosis in white but not black African RA patients. In contrast to these findings, the Arthritis Impact Measurement Scales tension score and non-steroidal anti-inflammatory agent use were associated with atherosclerosis in black Africans only. Additional studies by the same group found that black African women with RA had a 6.1 fold increased metabolic syndrome (MetS) prevalence compared to whites (Solomon A *et al.*, 2011; Dessein *et al.*, 2013b). In this regard, MetS triglycerides and the number of MetS criteria associated independently with plaque in white but, again, not in black African patients with RA. Also, excess adiposity was independently related to enhanced atherosclerosis in white by not black African women with RA (Solomon *et al.*, *2012*).

A reduced estimated glomerular filtration rate (eGFR) confers increased risk for adverse cardiovascular events (Salles e*t al.*, 2011). In the above mentioned cohort, a reduced eGFR was present in 49.1 and 30.6% of black and white Africans, respectively (Dessein *et al.*, 2015). Moreover, upon employing receiver operator characteristic curve analysis, 7 of 8 assessed eGFR equations predicted the presence of carotid plaque with sensitivities and specificities ranging from 42 to 60% and 70 to 91%, respectively, in black African RA patients. EGFR equations did however not accurately predict prevalent atherosclerosis amongst white Africans with RA. It is well recognized that the presence of carotid plaque confers increased CVD risk.

These findings are significant for black African patients with RA: first, they should no longer be considered immune to atherosclerosis; second, atherogenesis may currently consist of a different process in these patients; third, traditional and non-traditional cardiovascular risk factor evaluation as recommended in estimating the actual CVD risk in RA patients from developed populations should not be employed for the respective purpose in these patients; fourth, current optimal CVD risk stratification likely requires direct vascular imaging (e.g. carotid ultrasound) in order to determine CVD prevention by cardiovascular drugs, e.g. statins; fifth, the use of eGFR equations in CVD risk stratification is promising amongst black Africans who have no access to vascular imaging.

RA increases CVD risk to a similar extent as diabetes (Solomon *et al.*, 2010; Nurmohamed e*t al.*, 2010; Peters e*t al.*, 2010). In addition to traditional cardiovascular risk factors, systemic inflammation is strongly implicated in the enhanced atherosclerosis burden and CVD event rates associated with RA. Traditional cardiovascular risk factor based risk equations including the Framingham

score underestimate the actual CVD risk in RA. There is currently a need for alternative strategies in the elucidation of CVD risk and its optimal stratification in RA (Crownson & Gabriel, 2011; Dessein e*t al.*, 2013d). It is against this background that the need for identifying novel biomarkers of cardiovascular risk in this inflammatory disease is imperative (Crownson & Gabriel, 2011; Dessein e*t al.*, 2013d).

1.3 ADIPOKINES AS NOVEL BIOMARKERS

The role of adipose tissue in systemic metabolism and as a mediator of inflammatory changes is no longer disputed (Ouchi *et al.*, 2003; Berg & Scherer, 2005). Indeed, the importance of white adipose tissue as an endocrine organ that secretes bioactive molecules including hormones and proteins, has been recognised (Di Raimo *et al.*, 2015). White adipose tissue is predominantly present as subcutaneous and visceral adiposity comprising not only adipocytes but also pre-adipocytes, fibroblasts, vascular cells and immune cells (collectively referred to as stromal-vascular fraction (SVF) of adipose tissue) (Di Raimo *et al*, 2015). Protein secretion by adipocytes as well as SVF (adipose tissue), contributes to mechanisms of vascular insult and atheromatous changes (Ouchi *et al.*, 2011; Berg & Scherer, 2005). These soluble bioactive proteins that exhibit both pro- and anti-inflammatory effects, are referred to as adipo(cyto)kines (Scrivo e*t al.*, 2013; Funahashi e*t al.*, 1999). Trayhurn & Wood (2004) however recommend the use of the abbreviated term adipokine as a more accurate depiction of protein functionality and origin. Adipo(cyto)kine draws inference that the bioactive proteins secreted by adipose tissue are cytokines or cytokine-like which holds true in the case of IL-6 for instance but not for all of the adipokines (Trayhurn & Wood, 2004).

In the presence of obesity, hypertrophic adipocytes and stromal cells within the adipose tissue mediate systemic inflammatory changes that in turn effect a myriad of pathogenic pathways. The immunological activity state of adipose tissue is altered in the presence of obesity as mediated through changes in cellular composition (Ouchi e*t al.*, 2012). In addition to changes in cellular composition, the expression of adipokines varies depending on the site of adipose deposit (Samaras *et al.*, 2010; Fried e*t al.*, 1998). Circulating adipokine levels are thus representative of both the extent of adiposity as well as its biological activity (Dessein & Solomon, 2013).

Numerous adipokines have been identified and investigated in the past few decades. Complement factor D or the serine protease Adipsin, was the first adipokine to be identified (Cook *et al.*, 1987). This was followed by the identification of TNF as pro-inflammatory molecule that is secreted by adipose tissue and implicated in various inflammatory cascades in the presence of obesity (Ouchi e*t al.*, 2011; Hotamisligil e*t al.*, 1993). The current focus on adipose tissue as a bioactive endocrine organ was fuelled by the discovery of the cytokine-like hormone, leptin (Zhang *et al.*, 1994; Trayhurn & Wood, 2004). The subsequent discovery of adiponectin (ACRP30/ADIPOQ) played a key role in understanding the pathophysiology of obesity-linked disease cascades (Hu e*t al.*, 1996; Maeda e*t al*, 1996; Scherer e*t al.*, 1995).

In addition to TNF and IL-6, some of the most pertinent pro-inflammatory adipokines include leptin (Mantzoros CS, Farooqi *et al.*, 2002; Santos-Alvares *et al.*, 1999; Lord *et al.*, 1998), resistin (Jamaluddin *et al.*, 2012; Bokarewa *et al.*, 2005; Verma *et al.*, 2003), Retinal binding protein-4 (RBP-4) (Kotnik e*t al.*, 2011; Kloting e*t al.*, 2007; Balagopal *et al.*, 2007), chemerin (Yamawaki e*t al., 2*011; Rourke e*t al.*, 2013; Dessein e*t al.*, 2014d) and visfatin (Revollo e*t al.*, 2007; Moschen e*t al.*, 2007). Anti-

inflammatory adipokines include adiponectin and more recently identified SFRP5 (Ouchi e*t al.*, 2010).

These and other adipokines play a key roles in interactions between obesity, dyslipidemia, type 2 diabetes mellitus, hypertension and metabolic syndrome, all of which contribute to cardiovascular pathology (Berg & Scherer, 2005). Importantly in the present context, the involvement of adipokines in the pathophysiology of rheumatoid arthritis has been documented in numerous studies that alluded to both RA activity and severity (Gomez *et al.*, 2011).

Dessein et al evaluated the role of adipokines in a rheumatoid arthritis cohort within African context. The mentioned studies found that adiponectin concentrations were reduced in black RA patients of African descent. In these patients, adiponection concentrations were associated with favourable lipid concentrations and paradoxically with high blood pressure values (Dessein *et al.*, 2013a). In white RA patients, adiponectin concentrations were also paradoxically associated with increased endothelial activation (Dessein *et al.*, 2013e). These findings allude to the fact that adiponectin might exhibit altered expression and effects in the presence of a prototypic inflammatory disease such as rheumatoid arthritis. Varying concentrations of adiponectin in patients with RA could represent a compensatory mechanism proportional to CVD risk aimed at reducing this risk. Regardless of race, adiponectin related to a decreased presence of carotid plaque in RA patients amongst those without but not with deformed joints (Dessein e*t al.*, 2013a). The absence of the suggested adiponectin induced CVD risk protection may thus contribute to the reported link between RA severity and enhanced CVD risk in RA.

The adipokine resistin was associated with inflammation, endothelial activation and atherosclerosis (Dessein *et al*., 2013c; Dessein *et al*., 2014c).

In the presence of obesity, endothelial activation was mediated by leptin in young RA patients in the presence of obesity (Dessein *et al.*, 2014a). Dessein *et al.* employed sequential multivariate models, which revealed that obesity and chronic kidney disease induced atherosclerosis was mediated by leptin in RA.

Chemerin associated with angiopoietin 2 concentrations, which contribute to advanced atherosclerosis. Excess adiposity influenced the chemerin-atherosclerotic phenotype relations in RA (Dessein *et al.*, 2014d).

The pro-inflammatory adipokine RBP-4 was paradoxically associated with reduced endothelial activation but related to increased plaque prevalence in black and obese patients with RA (Dessein et al., 2014b).

The significance of these findings suggest that (1) adipokines contribute to RA related increased CVD risk; (2) adipokine concentrations comprise promising markers in CVD risk stratification in RA independent of traditional risk factors and disease activity; (3) targeting adipokines to treat RA can impact CVD risk; (4) data on adipokine production and adipokine-CVD relations can be RA specific; (5) the impact of adipokines on CVD risk in RA is pathophysiological context dependent, which calls for sensitivity analysis; (6) population origin impacts on adipokine-CVD relations.

Within the above context and in addition to the mentioned adipokines, Dessein et al further examined the impact of conventional compared to nonconventional cardiovascular risk factors including interleukin-6 levels on endothelial activation

patients with RA (Dessein e*t al.*, 2013a). Of the cardiovascular risk factors investigated, IL-6 contributed substantially to endothelial activation as estimated by an SD (z) score of endothelial activation molecule concentrations. This relationship was reproduced in various subgroups suggesting that the adipokine IL-6 can be employed in cardiovascular risk stratification in RA.

Taken together, reported evidence indicates that adipokines indeed mediate interactions amongst adiposity, systemic inflammation, RA activity and severity as well as CVD severity. This calls for the elucidation of more novel adipokines in the pathophysiology and enhanced CVD risk and its stratification associated with RA.

1.4 OMENTIN

The adipokine omentin, has recently received considerable attention as a critical mediator of metabolic and cardiovascular homeostasis. Omentin-1 (Intelectin-1, endothelial lectin HL-1, intestinal lactoferrin receptor, galactofuranose-binding lectin) was initially identified in small intestinal Paneth cells in 1998 (Komiya *et al.*, 1998). Omentin was implicated in pathogen recognition and gut defensive mechanisms against pathogenic bacterial translocation (Komiya *et al.*, 1998; Shaffler et al., 2005). This anti-inflammatory adipokine is predominantly expressed by the SVF of adipose tissue. Although omentin is produced in visceral rather than subcutaneous adipose tissue, in vitro studies suggest that omentin-1 exhibits insulin-sensitising effects in both visceral and subcutaneous adipose tissue. This is mediated through Akt/PKB phosphorylation in the absence of insulin (Yang *et al*., 2006).

Most reported evidence indicates that omentin protects against cardiometabolic risk (Tan *et al.*, 2010). As applies to adiponectin that was discussed earlier as anti-inflammatory and protective adipokine, omentin production is decreased in obesity (De Souza *et al.*, 2007). Reduced omentin concentrations are associated with metabolic risk factors including impaired glucose metabolism, low HDL-cholesterol levels and hypertension (Pan e*t al.*, 2010; Moreno-Navarrete e*t al.*, 2010; Saremi e*t al.*, 2010; Shibata e*t al.*, 2012). Omentin induces vasodilation through endothelial-derived nitric oxide production (Yamawaki e*t al.*, 2010), and decreases tumor necrosis factor-α induced endothelial activation (Zhong e*t al.*, 2012). Reduced omentin concentrations are associated with arterial stiffness (Yoo e*t al.*, 2011), atherosclerosis (Shibata e*t al.*, 2011; Liu e*t al.*, 2011; Kadoglou e*t al.*, 2014), plaque vulnerability (Kadoglou e*t al.*, 2014; De Jager et al., 2016), established coronary artery disease presence and severity (Shang e*t al.*, 2011; Zhong e*t al.*, 2011; Shibata e*t al.*, 2011), diastolic dysfunction (Greulich e*t al.*, 2013) and, disease severity and incident cardiovascular event rates amongst patients with heart failure (Namuri e*t al.*, 2014).

As previously alluded to, concentrations of adipokines other than omentin are related to inflammation, traditional cardiovascular risk factors and subclinical CVD in RA (Gonzalez-Gay *et al.*, 2008; Gonzalez-Gay e*t al.*, 2009; Dessein e*t al.*, 2013a; Dessein e*t al.*, 2013c; Dessein e*t al.*, 2013e; Dessein e*t al.*, 2014a; Dessein e*t al.*, 2014b; Dessein *et al.*, 2014c; Dessein *et al.*, 2014d; Dessein *et al.*, 2014e; Popa e*t al.*, 2009; Rourke e*t al.*, 2013). Cellular studies revealed that several adipokines participate in the pathophysiology of RA (Gomez e*t al.*, 2011; Eisinger e*t al.*, 2012). Synovial fluid levels of omentin were reduced in RA compared to osteoarthritis patients (Senolt e*t al.*, 2010). Reduced expression of omentin in omental adipose

tissue may contribute to transmural intestinal inflammation in Crohn's disease (Shaffler e*t al.*, 2005). Omentin decreases vascular inflammation (Yamawaki *et al.*, 2011). These findings suggest that omentin has anti-inflammatory properties (Lemieux e*t al.*, 2001). Whether omentin can contribute to the enhanced CVD risk and its stratification in RA awaits investigation.

1.5 STUDY OBJECTIVES

In the present study, we therefore explored the independent relationships of omentin concentrations with metabolic risk factors, endothelial activation, atherosclerosis and matrix metalloproteinases (MMP) 2, 3 and 9 that mediate altered plaque vulnerability to rupture (Back *et al.*, 2010; Galis e*t al.*, 1994; Beaudeux e*t al.*, 2003; Samnegard e*t al.*, 2006; Wu e*t al.*, 2005; Schoenhagen e*t al.*, 2002; Inoue e*t al.*, 2003), in patients with RA. We also determined the potential influence of population origin, disease activity and severity and systemic inflammation on the respective relations.

CHAPTER 2

MATERIALS AND METHODS

2.1 PATIENTS

This study was performed according to the principles outlined in the Helsinki declaration and was approved by the Human Research Ethics Committee (Medical) (approval number: M12-05-62) from the University of the Witwatersrand, Johannesburg, South Africa, and forms part of an ongoing investigation on cardiovascular risk in RA patients from a public and private health care center (Dessein e*t al.*, 2013a; Dessein *et al.*, 2013c; Dessein *et al.*, 2013e ;Dessein *et al.*, 2014a; Dessein *et al.*, 2014b; Dessein *et al.*, 2014c; Dessein *et al.*, 2014d; Dessein *et al.*, 2014e). A total of 213 (104 black, 109 white) patients that met the 1988 American College of Rheumatology (ACR) and 2012 ACR/European League Against Rheumatism criteria for RA participated (Arnett *et al.*, 1988; Aletaha *et al.*, 2010). Informed, written consent was obtained from all participants.

2.2 ASSESSMENTS

Baseline characteristics and conventional metabolic risk factors were recorded using previously described methods (Dessein e*t al.*, 2013a; Dessein *et al.*, 2013c; Dessein *et al.*, 2013e ;Dessein *et al.*, 2014a; Dessein *et al.*, 2014b; Dessein *et al.*, 2014c; Dessein *et al.*, 2014d; Dessein *et al.*, 2014e). Briefly, demographic features and anthropometric measures including height, weight, waist and hip circumference were recorded according to standard protocols. Body mass index (BMI) and waist circumference were used as indicators of generalized adiposity and fat distribution, respectively. RA activity was evaluated by the Clinical Disease Activity Score (CDAI). Disease severity markers included the number of deformed joints, rheumatoid factor (RF) status and current or previously recorded (hospital record

review) extra-articular manifestations that were defined as the presence of pericarditis, pleuritis, Felty's syndrome, cutaneous vasculitis, neuropathy, scleritis or episcleritis, retinal vasculitis, glomerulonephritis, vasculitis affecting other organs, amyloidosis, keratoconjunctivitis, xerostomia, Sjorgen's syndrome, pulmonary fibrosis, bronchiolitis obliterans organizing pneumonia, cervical myelopathy, subcutaneous nodules and rheumatoid nodules in other locations. C-reactive protein (CRP) concentrations were evaluated by immunoturbidimetric methods. We measured IL-6 concentrations using a solid-phase sandwich enzyme-linked immunosorbant assay (ELISA) (Quantikine HS, R&D Systems, Inc., Minneapolis, MN, USA). The lower detection limit ranged from 0.02 to 0.11 pg/mL and the inter- and intra-assay coefficients of variation were 7.8 and 7.4%, respectively. Standard laboratory tests of erythrocyte sedimentation rate, renal and liver function, hematological variables, lipids and glucose were performed. The glomerular filtration rate (GFR) was estimated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation (Dessein *et al.*, 2015). Use of synthetic disease modifying agents and cardiovascular drugs were recorded.

Evaluated conventional metabolic risk factors comprised systolic and diastolic blood pressures, high-density lipoprotein, low-density lipoprotein and triglyceride concentrations and lipid ratios, as well as serum glucose levels. Hypertension was defined as an average systolic blood pressure ≥ 140 mmHg and/or diastolic blood pressure of ≥ 90 mmHg and/or current use of antihypertensive medications. Patients were diagnosed as having dyslipidemia when the cholesterol/HDL ratio was > 4 (Peters *et al.*, 2010). Participants were identified as diabetic when their fasting plasma glucose concentration was ≥ 7 mmol/l or when using glucose lowering agents.

The concentrations of six adipokines other than omentin 1 were also measured and included total and high molecular weight adiponectin, leptin, chemerin, retinol-binding protein 4 (RBP-4) and resistin as previously described by us (Dessein e*t al.*, 2013a; Dessein *et al.*, 2013c; Dessein *et al.*, 2013e ;Dessein *et al.*, 2014a; Dessein *et al.*, 2014b; Dessein *et al.*, 2014c; Dessein *et al.*, 2014d; Dessein *et al.*, 2014e).

Early endothelial activation molecule concentrations were assessed, including those of soluble E-selectin, vascular cell adhesion molecule 1 (VCAM-1), intercellular adhesion molecule 1 (ICAM-1), monocyte chemoattractant protein 1 (MCP-1), using solid phase sandwich ELISA (QuantikineHS). The lower detection limits were 0.009 ng/l, 0.6 ng/l, 0.096 ng/l and 5.0 pg/ml, respectively; their interassay and intraassay coefficients of variation were 7.9 and 5.8, 7.0 and 3.1, 5.5 and 4.6, and 5.7 and 5.8%, respectively. Also, the levels of 2 further endothelial activation markers including asymmetric dimethylarginine (ADMA) and angiopioetin 2 were evaluated, with lower detection limits of 0.05 µmol/l and 1.2 pg/ml, respectively; their interassay and intraassay coefficients of variation were 7.8 and 8.8, and 8.9 and 5.9%, respectively.

Carotid artery ultrasound (US) measurements were conducted by two operators. Images of at least 1cm length of the distal common carotid arteries were obtained for measurement of the carotid intima media thickness, using the optimal angle of incidence (defined as the longitudinal angle of approach where both branches of the internal and external carotid artery are clearly visible (Gepner *et al.*, 2006). Carotid intima media thickness measurements were made with high-resolution B-mode US (Image Point, Hewlett Packard and SonoCalc IMT, Sonosite Inc.), using linear array 7.5 MHz probes. The US methods utilized in the private sector have been previously published in detail (Dessein *et al.*, 2007). The public sector equipment employed

specialized software allowing semi-automated border detection, which was previously found to provide highly reproducible results (Gepner e*t al.*, 2006). The c-IMT measurement was recorded as the mean of the left and right carotid artery values. Carotid artery plaque was defined as a focal structure that encroaches into the arterial lumen of at least 0.5mm or 50% of the surrounding intima-media thickness value, or demonstrates a thickness of > 1.5 mm as measured from the media-adventitia to the intima-lumen interface (Touboul e*t al.*, 2007). Both research operators were blinded to patient cardiovascular risk profiles. Repeat US examinations on 23 patients from either public or private sectors confirmed an intraobserver coefficient of variation of 5.8% and 4.1% for private and public healthcare patients, respectively, and an interobserver coefficient of variation of 8.0% for measurements made by two operators. Both operators identified carotid artery bulb and/or internal carotid artery plaque in 11 of these 23 patients, with full agreement.

MMP-2, -3 and -9 concentrations were quantified. Their lower detection limits were 3.5 ng/ml, 0.3 pg/ml and 10 pg/ml, respectively; the interassay and intraassay coefficients of variation were 10.0 and 12.0% for each of the 3 measurements.

Omentin-1 concentrations were determined by a solid-phase sandwich ELISA (QuantikineHS). The lower detection limit was 0.4 ng/ml.

2.3 DATA MANAGEMENT AND ANALYSIS

Continuous variables were expressed as mean (SD), or median (interquartile range) when non-normally distributed. Non-normally distributed characteristics were also

logarithmically transformed prior to their inclusion in multivariable statistical analysis. Dichotomous variables were expressed as proportions or percentages.

The relationship of age at disease onset, age at time of the study, gender and population grouping with omentin concentrations were determined in a mixed regression model. Associations of other baseline characteristics with omentin levels were assessed in demographic characteristic adjusted models.

The independent relationships of omentin levels with metabolic risk factors, endothelial activation molecule concentrations, atherosclerosis and MMP levels were evaluated in multivariate linear of logistic models with adjustment for potential confounders and/or mediators as identified in the previous analysis.

Amongst patients with RA, it is those with severe disease that are particularly at high risk for CVD (Dessein *et al.*, 2007; Solomon *et al.*, 2010; Nurmohamed e*t al.*, 2010; Peters e*t al.*, 2010). Further, adipokine effects on cardiovascular risk depend on pathophysiological context (Gonzalez-Gay *et al.*, 2008; Gonzalez-Gay e*t al.*, 2009; Dessein e*t al.*, 2013a; Dessein e*t al.*, 2013c; Dessein e*t al.*, 2013e; Dessein e*t al.*, 2014a; Dessein e*t al.*, 2014b; Dessein *et al.*, 2014c; Dessein *et al.*, 2014d; Dessein *et al.*, 2014e; Popa e*t al.*, 2009; Rourke e*t al.*, 2013), which includes not only disease activity and severity as well as systemic inflammation but also adiposity status and population origin in RA patients (Dessein e*t al.*, 2013e; Dessein e*t al.*, 2014b). We therefore assessed the impact of the respective patient characteristics on omentin concentration-subclinical CVD relations by adding the appropriate interaction terms and their individual components to the models, and subsequent stratified analysis, that is, in subgroups with and without patients characteristics of interest, when indicated (significant interaction p values). For this purpose, patients with a BMI of ≥

30 kg/m^2 and those who met the National Cholesterol Education Program for metabolic syndrome (MetS) waist criterion (Dessein *et al.*, 2006) were considered to sustain overall and abdominal obesity, respectively. When appropriate, patients were categorized in subgroups based on median values.

Statistical computations were made using IBM SPSS statistics (version 22.0, IBM, USA). Statistical significance was set as $p < 0.05$.

CHAPTER 3

RESULTS

3. RESULTS

Descriptive statistics of recorded patient characteristics are detailed in Table 1. The mean (±SD) age at time of the study was 56.9 (11.1) years with a mean disease duration of 13.5 (9.5) years. The median (interquartile range) CDAI was 7 (Solomon *et al.*, 2010; Nurmohamed e*t al.*, 2010; Peters e*t al.*, 2010; Crowson & Gabriel, 2011; Dessein e*t al.*, 2013d; Komiya e*t al.*, 1998; Shaffler *et al.*, 2005; Yang e*t al.*, 2006; Lemieux e, 2001; Tan *et al.*, 2010; De Souza e*t al.*, 2007; Pan e*t al.*, 2010; Moreno-Navarrete *et al.*, 2010). All patients received synthetic disease modifying agents. Of the participants, 57.3 % were hypertensive and 40.3 % had carotid plaque.

TABLE 1. RECORDED CHARACTERISTICS IN 213 PATIENTS WITH RA.

Demographic characteristics	
Age at disease onset, yrs	43.4 (13.4)
Age at study time, yrs	56.9 (11.1)
Female sex	83.1
Black	48.8
White	51.2
Lifestyle factors	
Exercise	36.8
Alcohol use	21.1
Current smoking	7.5
Anthropometry	
Body mass index, kg/m^2	27.4 (5.9)
Waist circumference, cm	91 (13)
Waist-hip ratio	0.86 (81-91)
Cardiovascular agents	
Antihypertensives	46.9
Statins	27.2
Ezetimibe	0.9
Oral glucose-lowering agents	8.5
Insulin	1.4
RA characteristics	
Disease duration, yrs	13.5 (9.5)
Rheumatoid factor-positive	75.5
Clinical Disease Activity Index	7 (2-14)
Erythrocyte sedimentation rate, mm/h	11 (5-26)
C-reactive protein, mg/l	5.3 (2.2-13)
Interleukin 6, pg/ml	3.6 (2.2-6.0)

Leukocytes, n/nl	3.8 (1.4)
N deformed joints	9 (9)
Extraarticular manifestations	7.5
Synthetic disease-modifying agents	
Methotrexate	84.0
Chloroquine	66.7
Leflunomide	31.0
Sulfasalazine	18.3
Azathioprine	15.0
Tetracycline	12.7
Cyclophosphamide	3.8
Penicillamine	3.3
Number	2.4 (0.9)
Prednisone use	2.3
Tumor necrosis factor-α blockade	3.8
CKD-EPI, ml/min/1.73 m^2	97 (82-116)
Conventional Metabolic Risk Factors	
Hypertension	57.3
Systolic blood pressure, mmHg	133 (21)
Diastolic blood pressure, mmHg	82 (12)
Total cholesterol, mmol/l	4.8 (1.0)
HDL cholesterol, mmol/l	1.52 (1.3-1.90)
LDL cholesterol, mmol/l	2.7 (0.8)
Triglycerides, mmol/l	1.0 (0.8-1.4)
Cholesterol-HDL cholesterol ratio	3.2 (0.9)
Cholesterol-HDL cholesterol ratio>4	16.8
Non-HDL cholesterol, mmol/l	3.8 (1.0)
Diabetes	11.7
Glucose, mmol/l	4.7 (4.4-5.2)

Adipokines	
Leptin, ng/ml	10.5 (5.5-18.4)
Chemerin, ng/ml	114 (35)
Total adiponectin, µg/ml	7.3 (4.8-12.3)
High molecular adiponectin, µg/ml	3.4 (1.8-5.9)
Resistin, ng/ml	37.4 (22.9-56.8)
Retinol binding protein 4, ng/ml	37.6 (20.4)
Omentin-1, ng/ml	9.0 (5.5-18.4)
Endothelial activation molecules	
E-selectin, ng/ml	38.5 (18.3)
VCAM-1, ng/ml	834 (674-1037)
ICAM-1, ng/ml	275 (206-353)
MCP-1, pg/ml	421 (261-680)
Angiopoietin 2, pg/ml	2.57 (2.08-3.36)
ADMA, µmol/l	0.63 (0.51-0.80)
Plaque vulnerability	
MMP-2, ng/ml	1,430 (624-2,435)
MMP-3, pg/ml	10.2 (3.5-22.5)
MMP-9, ng/ml	4,606 (3,446-6,570)
Atherosclerosis	
Intima-media thickness, mm	0.709 (0.112)
Plaque	40.3

Dichotomous variables are expressed as proportions or percentages and continuous characteristics as mean ± SD or median (interquartile range). RA: rheumatoid arthritis; N: number of CKD-EPI: Chronic Kidney Disease Epidemiology Collaboration; HDL: high-density lipoprotein; LDL: low-density lipoprotein; VCAM-1: vascular adhesion molecule 1; ICAM-1: intercellular adhesion molecule 1; MCP-1: monocyte chemoattractant protein 1; ADMA: asymmetric dimethylarginine; MMP: matrix metalloproteinase.

Associations of baseline characteristics with omentin concentrations.

Table 2 gives the associations of baseline characteristics with omentin concentrations at $p<0.15$, which were included in subsequent analysis as potential confounders. The CKD-EPI was significantly related to omentin levels.

Independent relationships of omentin concentrations with metabolic risk factors. Omentin concentrations were not related to any of the conventional metabolic risk factors or adipokine levels (data not shown).

Independent associations of omentin concentrations with endothelial activation molecule concentrations, atherosclerosis and matrix metalloproteinase levels.

As shown in Table 3, omentin levels were inversely associated with those of matrix metalloproteinase-3 in both univariate (ß (SE) = -0.373 (0.122), p=0.003) and multivariate (ß (SE) = -0.364 (0.113), p=0.002) analysis. In a separate model in which CRP concentrations (disease activity) and the deformity joint count (disease severity) were additionally adjusted for, omentin levels remained strongly related to those of matrix metalloproteinase-3 (ß (SE) = -0.378 (0.118), p=0.002). Also, additional adjustment for the Framingham score (overall conventional CVD risk burden) or waist circumference and BMI (adiposity) did not alter the respective finding (ß (SE) = -0.362 (0.116), p=0.002 and ß (SE) = -0.334 (0.112), p=0.003, respectively). Omentin concentrations were not related to those of endothelial activation molecules and cIMT and plaque presence.

TABLE 2. ASSOCIATIONS OF BASELINE CHARACTERISTICS WITH OMENTIN CONCENTRATIONS AT P < 0.15.

Characteristics	ß (SE)	p
Demographic characteristics		
Age at time of the study	0.000 (0.002)	0.8
Age at disease onset	-0.002 (0.002)	0.2
Female sex	-0.032 (0.060)	0.6
Black	0.035 (0.045)	0.4
Lifestyle factors		
Alcohol use	0.096 (0.060)	0.1
Smoking	0.156 (0.083)	0.06
Cardiovascular agents		
Antihypertensives	-0.075 (0.046)	0.1
RA characteristics		
CDAI*	-0.081 (0.048)	0.09
Synthetic disease-modifying agents		
Methotrexate	0.107 (0.061)	0.08
Azathioprine	-0.116 (-0.061)	0.06
Tumor necrosis factor-α blockade	0.172 (0.117)	0.1
Glomerular filtration rate (CKD-EPI)	**-0.003 (0.001)**	**0.04**

ß: regression coefficients; SE: standard error; VCAM-1: vascular adhesion molecule 1; CDAI: Clinical Disease Activity Index; CKD-EPI: Chronic Kidney Disease Epidemiology Collaboration; *logarithmically transformed; significant associations (values) are shown in bold.

TABLE 3. RELATIONSHIPS OF OMENTIN CONCENTRATIONS WITH ENDOTHELIAL ACTIVATION, MATRIX METALLOPROTEINASES AND ATHEROSCLEROSIS.

	Univariate		**Multivariate**	
Characteristics	**ß (SE)**	**p**	**ß (SE)**	**p**
EA molecules				
E-selectin	-5.530(4.186)	0.2	-5.932(4.509)	0.2
VCAM-1*	-0.016(0.034)	0.6	-0.027(0.037)	0.5
ICAM-1*	-0.004(0.040)	0.9	0.007(0.041)	0.9
MCP-1	0.049(0.064)	0.4	0.084(0.068)	0.2
Angiopoietin 2*	0.052(0.044)	0.2	-0.058(0.045)	0.2
ADMA	0.039 (0.036)	0.3	0.047(0.038)	0.2
Plaque vulnerability markers				
MMP-2*	0.081 (0.108)	0.5	0.024(0.113)	0.8
MMP-3*	**-0.373 (0.122)**	**0.003**	**-0.364(0.113)**	**0.002**
MMP-9*	0.068 (0.054)	0.2	0.083(0.056)	0.2
Atherosclerosis				
Intima-media thickness	-0.150 (0.024)	0.5	-0.008(0.024)	0.7
	OR(95% CI)	**p**	**OR(95% CI)**	**p**
Plaque	1.26(0.52-3.04)	0.6	1.14(0.41-3.18)	0.3

Associations were assessed in age at disease onset, race, gender, alcohol use, smoking, glomerular filtration rate (CKD-EPI), CDAI, methotrexate and azathioprine use and tumor necrosis factor-α blockade adjusted models.
ß: regression coefficients; SE: standard error; EA: endothelial activation; VCAM-1: vascular adhesion molecule 1; ICAM-1: intercellular adhesion molecule 1; MCP-1: monocyte chemoattractant protein 1; ADMA: asymmetric dimethylarginine; MMP: Matrix metalloproteinase; *logarithmically transformed; significant associations (values) are shown in bold.

Impact of patient characteristics on omentin concentration - subclinical CVD relations.

The potential impact of patient characteristics (see data analysis) on omentin concentration-endothelial activation, -atherosclerosis and -plaque vulnerability marker relations, were systematically explored. Where significant interactions were noted, subsequent analysis in patients with and without the characteristics of interest was performed.

Table 4 shows that amongst patient characteristics, CDAI, erythrocyte sedimentation rate (ESR), deformed joint count, generalized and abdominal adiposity and population origin each impacted on the omentin concentration-MMP 3 level relations. As also given in Table 4, this translated into consistent disparities in omentin concentration-MMP-3 relations amongst the respective subgroups in stratified analysis. Thus, omentin levels were related to those of MMP-3 in patients without moderate or high disease activity (CDAI > 10) and a large ESR or deformed joint count. Additionally, omentin concentrations were associated with those of MMP-3 in patients without but not with generalized or abdominal obesity and in white but not black African patients with RA. CRP levels did not significantly impact the omentin-MMP-3 concentration relation (interaction p = 0.1). Nevertheless, omentin concentrations further related to those of MMP-3 in the 104 patients with a CRP level of $\leq$ 5.3 mg/l (median value in all patients) but not in the 103 with a CRP concentration of > 5.3 mg/l (β (SE) = -0.459 (0.147), p = 0.002 and β (SE) = -0.309 (0.198), p = 0.1, respectively).

The CDAI impacted on the omentin concentration-cIMT relation (interaction p = 0.02). However, as applied to the analysis amongst all patients, omentin

concentrations remained unrelated to cIMT in patients with (β (SE) = -0.034 (0.032), p=0.3) and without (β (SE) = 0.011 (0.038), p=0.8) a CDAI value of > 10.

TABLE 4. INDEPENDENT RELATIONSHIPS OF OMENTIN CONCENTRATIONS WITH MMP-3 IN SUBGROUPS

		MMP-3		Interactions
Subgroups	**Number**	**ß (SE)**	**p**	**P**
Population				
White	109	**-0.450 (0.153)**	**0.0004**	
Black	104	-0.099 (0.195)	0.6	**0.009**
BMI >29.9 kg/m^2				
No	144	**-0.355 (0.124)**	**0.005**	
Yes	64	-0.540 (0.334)	0.1	**0.02**
Missing	5			
MetS waist				
No	114	**-0.454 (0.142)**	**0.002**	
Yes	96	-0.080 (0.217)	0.7	**0.02**
Missing	3			
CDAI >10				
No	127	**-0.411 (0.139)**	**0.004**	
Yes	85	-0.286 (0.202)	0.2	**0.04**
Missing	1			
ESR >11 mm/hr				
No	105	**-0.534 (0.161)**	**0.001**	
Yes	102	-0.212 (0.168)	0.2	**0.04**
Missing	6			
N Deformed joints >7				
No	111	**-0.554 (0.165)**	**0.001**	
Yes	101	-0.110 (0.173)	0.5	**0.007**
Missing	1			

Associations were assessed in age at disease onset, race, gender, alcohol use, smoking, compromised glomerular filtration rate (CKD-EPI), CDAI and methotrexate and azathioprine use and tumor necrosis factor-α blockade adjusted models. MMP: matrix metalloproteinase; ß: regression coefficients; SE: standard error; BMI: body mass index; MetS: metabolic syndrome; CDAI: Clinical Disease Activity Index;

ESR: erythrocyte sedimentation rate; *logarithmically transformed; significant associations(values) are shown in bold.

CHAPTER 4

DISCUSSION

The present study documents that omentin concentrations are strongly and inversely associated with those of MMP-3 in patients with RA. MMP-3 expression is altered in obesity (Traurig e*t al.*, 2006) and its circulating concentrations can represent disease activity (Keyszer e*t al.*, 1999) and joint damage (Yamanaka *et al.*, 2000) in RA. The omentin-MMP-3 concentration relation in the present investigation was independent of adiposity measures and the CDAI and deformity joint count as well as a large number of other potential confounders.

Matrix metalloproteinases are endopeptidases that mediate remodeling of the extracellular matrix (Back *et al.*, 2010). MMP-3 is highly expressed in the shoulders (of plaques) and regions of foam cell accumulation of atherosclerotic plaques (Galis *et al.*, 1994). Enhanced MMP-3 expression colocalizes with casein lysis in atherosclerotic lesions, which supports its increased activity (Galis *et al.*, 1994). Circulating MMP-3 concentrations are increased in patients with atherosclerosis (Beaudeux *et al.*, 2003). Reported evidence indicates that MMP-3 may particularly contribute to the progression of atherosclerosis (Samnegard *et al.*, 2006). Against this background, our present findings indicate that although omentin concentrations are not related to prevalent atherosclerosis in RA as in the non-RA population (Shibata *et al.*, 2011; Liu & Wang *et al.*, 2011; Kadoglou *et al.*, 2014), this molecule may nevertheless contribute to its acceleration, which is well recognized in this disease (Dessein *et al.*, 2007; Solomon *et al.*, 2010; Nurmohamed e*t al.*, 2010; Peters e*t al.*, 2010). Future longitudinal investigations are needed to address this possibility.

MMP-3 levels also predict incident cardiovascular event rates amongst patients with stable coronary artery disease (Wu e*t al.*, 2005). MMP-3 is overexpressed in coronary atherosclerotic lesions that exhibit positive arterial remodelling, which is

associated with unstable clinical presentation (Schoenhagen e*t al.*, 2002). Although reduced circulating MMP-3 concentrations were recently reported in patients with acute coronary syndrome (ACS) (Samnegard e*t al.*, 2006), circulating MMP-3 levels in ACS patients may not represent local concentrations at the site of plaque rupture. Thus, in blood withdrawn from the coronary sinus but not aorta, MMP-3 concentrations were actually larger in ACS patients compared to those with stable coronary disease and controls (Inoue e*t al.*, 2003). Plaque vulnerability is increased and related to disease activity in RA (Aubry *et al.*, 2007; Stamatelopoulos et al, 2007). These reported findings together with our current results suggest that omentin can enhance plaque vulnerability to rupture in RA as reported in non-RA subjects (Kadoglou e*t al.*, 2014).

Importantly in the context of RA, we found that disease activity and severity and the ESR consistently modified the omentin-MMP-3 relation in RA. Indeed, we have previously reported that similar to in non-RA subjects, the potential impact of adipokines on CVD risk is frequently influenced by disease characteristics in RA (Dessein e*t al.*, 2013a; Dessein *et al.*, 2013c; Dessein *et al.*, 2013e ;Dessein *et al.*, 2014a; Dessein *et al.*, 2014b; Dessein *et al.*, 2014c; Dessein *et al.*, 2014d; Dessein *et al.*, 2014e). Our present results show that the potential beneficial effects of omentin on CVD risk are lost in patients with moderate and marked RA activity, a high ESR value and extensive joint damage. An abrogated impact of omentin may therefore contribute to the recognized increased CVD risk associated with severe RA (Dessein *et al.*, 2007; Solomon *et al.*, 2010; Nurmohamed e*t al.*, 2010; Peters e*t al.*, 2010). This has implications in CVD risk management through adequate RA activity and inflammation control.

The impact of excess adiposity on CVD risk in RA is controversial at present (Kitas & Gabriel, 2011; Stavropoulos *et al.*, 2011). Nevertheless, in our setting, excess adiposity increases cardiovascular risk in patients with RA (Solomon e*t al.*, 2011). Congruently, our current analysis disclosed that the potentially protective effects of omentin against CVD are also lost in RA patients with generalized or abdominal obesity. Whether management of obesity can reduce CVD risk by altering omentin effects in RA merits further investigation.

Population origin was not associated with altered omentin concentrations. However, contrary to our findings in white African participants with RA, black patients did not experience an omentin concentration related decrease in MMP-3. Disparities in the associations of polymorphisms in adipokine related genes with subclinical CVD amongst black and white participants were previously documented in the multi-ethnic study of atherosclerosis (Wassel e*t al.*, 2011). These findings and our current results reinforce the need for population specific CVD risk stratification (Solomon e*t al.*, 2014).

The omentin-MMP-3 concentration relationship was also independent of the Framingham score, which predicts severe or high risk atherosclerosis to a clinically useful extent in RA (Dessein e*t al.*, 2016). Consideration of omentin concentrations may enhance CVD risk stratification in RA.

In the present study, the estimated glomerular filtration rate comprised the only baseline characteristic that was significantly and inversely associated with omentin concentrations. High omentin concentrations were previously documented in non-RA patients with renal impairment (Sengul e*t al.*, 2013). Notably, the associations of omentin concentrations with conventional metabolic risk and levels of other

adipokines that were disclosed in non-RA subjects (Pan e*t al.*, 2010; Moreno-Navarrete e*t al.*, 2010; Saremi e*t al.*, 2010; Shibata e*t al.*, 2012) were not reproduced in our patients with RA. This argues against the indiscriminate extrapolation of reported findings on the role of omentin in reduced metabolic CVD risk in the non-RA population, to patients with RA.

We recently reported that adiponectin, leptin, resistin, retinol binding protein-4 and chemerin levels were each independently related to endothelial activation and atherosclerosis extent in RA (Dessein e*t al.*, 2013a; Dessein *et al.*, 2013c; Dessein *et al.*, 2013e ;Dessein *et al.*, 2014a; Dessein *et al.*, 2014b; Dessein *et al.*, 2014c; Dessein *et al.*, 2014d; Dessein *et al.*, 2014e). Interestingly, these findings contrast to our current results obtained in all patients as well as in various subgroups. The potential impact of adipokines other than omentin on MMP concentrations has, to our knowledge, not been reported to date.

This investigation comprises comprehensively assessed RA patients. Apart from previously mentioned limitations, our coss-sectional study design precludes drawing inferences on the direction of causality. Although omentin is overall considered to reduce CVD risk (Shaffler e*t al.*, 2005; Yang e*t al.*, 2006; Tan *et al.*, 2010; De Souza e*t al.*, 2007; Pan e*t al.*, 2010; Moreno-Navarrete e*t al.*, 2010; Saremi e*t al.*, 2010; Shibata e*t al.*, 2012; Yamawaki e*t al.*, 2010; Zhong e*t al.*, 2012; Yoo e*t al.*, 2011; Shibata e*t al.*, 2011a; Liu e*t al.*, 2011; Kadoglou e*t al.*, 2014; Shang e*t al.*, 2011; Zhong e*t al.*, 2011; Shibata e*t al.*, 2011b; Greulich e*t al.*, 2013; Namuri e*t al.,* 2014), in a recent prospective study in 295 patients with established or suspected stable coronary artery disease, omentin concentrations were directly related to incident cardiovascular events (Saely e*t al.*, 2016). This may have represented a paradoxical and compensatory alteration in omentin production because of prevalent CVD and

aimed at reducing this risk. Indeed, similar findings on the potential effects of adiponectin on CVD risk were previously reported in both RA and non-RA subjects (Dessein *et al.*, 2013a; Dessein *et al.* 2013e; Sattar & Nelson, 2008).

In conclusion, omentin concentrations do not represent endothelial activation and atherosclerosis extent in RA. However, omentin concentrations were inversely associated with those of MMP-3, a surrogate marker of plaque vulnerability to rupture, in white but not black Africans with RA. This inverse relationship was also absent RA patients with moderate or severe RA activity and large ESR values and joint deformity counts. A loss of beneficial effects of omentin on plaque instability may contribute to the link between severe disease and increased cardiovascular risk in RA. The role of omentin in plaque vulnerability and CVD risk stratification amongst patients with RA merits future longitudinal investigation.

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