Abstract

Rheumatoid arthritis (RA) is a chronic inflammatory condition that confers an increased risk for the development of cardiovascular disease, independent of conventional cardiovascular risk factors. Systemic high-grade inflammation is a key determinant in the development of cardiovascular disease. The mechanisms whereby RA contributes to subclinical cardiovascular disease is under investigation. Adipokines are intricately linked to the regulation of various inflammatory processes and may be a mechanistic link to subclinical cardiovascular disease. Several adipokines are altered in RA compared to the general population. Whether adipokines are reliable biomarkers of subclincial cardiovascular disease in RA warrant investigation. This thesis thus focused on exploring the contribution of the adipokines nesfatin, visfatin and vaspin to the development of subclinical cardiovascular disease, including atherosclerosis, arterial stiffness and cardiac function, in patients with RA.

Atherosclerosis contributes largely to the increased cardiovascular disease mortality in RA. Nesfatin and visfatin concentrations are altered in RA compared to the general population. Nesfatin is an anti-inflammatory molecule and is associated with reduced risk of atherosclerosis in the general population. In contrast, visfatin concentrations are increased in chronic inflammatory conditions and are directly associated with atherosclerosis. The first study examined the potential impact of nesfatin and visfatin on metabolic risk factors, endothelial activation, atherosclerosis and plaque vulnerability mediators in 232 patients with established RA. Adipokine concentrations, endothelial activation marker concentrations including E-selectin, vascular adhesion molecule-1, intracellular adhesion molecule-1, monocyte chemoattractant protein-1, angiopoietin-2 and asymmetric dimethylarginine and plaque stability mediators, including matrix metalloproteinases (MMP) 2, 3 and 9 were assessed by ELISA. Common carotid intima-media thickness (cIMT) and carotid artery plaque were assessed by ultrasound. Independent associations of nesfatin and visfatin concentrations with metabolic risk factors, endothelial activation, carotid atherosclerosis and altered plaque stability were determined in multivariate regression analysis. Rheumatoid factor (RF) positivity was associated with nesfatin (β (SE) = 0.65 (0.14), $p<0.0001$) and visfatin levels (β (SE) = 0.16 (0.07), $p = 0.03$). Visfatin concentrations were related to diastolic blood pressure (β (SE) = 4.54 (1.70), $p = 0.01$) and diabetes (β (SE) = 0.09 (0.05), $p = 0.04$). Nesfatin levels were associated with cIMT (β (SE) = -0.02 (0.01), $p = 0.007$). Nesfatin (β (SE) = 0.12 (0.03), $p = 0.001$) and visfatin concentrations (β (SE) = 0.23 (0.07), $p = 0.001$) were related to those of matrix metalloproteinase-2 (MMP-2), a plaque stability mediator. The nesfatin-MMP-2 and visfatin-MMP-2 relations were stronger in RF negative compared to RF positive patients. This study showed that nesfatin was associated with reduced atherosclerosis and increased plaque stability mediator levels in RA. Visfatin was related to adverse cardio-metabolic risk in RA. Increased MMP-2

expression in relation to visfatin may represent a compensatory mechanism aimed at reducing cardiovascular risk in RA.

Vaspin concentrations are also altered in RA. Vaspin concentrations have been associated with atherosclerotic changes and have been identified as an independent prognostic marker of adverse cardiac events. The second study examined the potential impact of vaspin on metabolic risk factors, endothelial activation, carotid atherosclerosis, and plaque vulnerability mediators in 170 RA patients. Relationships between vaspin and endothelial activation, plaque vulnerability mediators and atherosclerosis were identified in multivariate regression analysis. Vaspin concentrations were associated with RF positivity (β (SE) = 0.03 (0.10), p = 0.002). Vaspin was not associated with any cardiovascular disease markers, endothelial activation, or atherosclerosis in both univariate and multivariate analysis. The relationship between MMP-2 and vaspin concentrations were, however, influenced by the presence of major cardiovascular risk factors (β (SE) = -0.08 (0.03), p = 0.02). In subgroup analysis, vaspin levels were positively associated with those of MMP-2 in patients with no major cardiovascular risk factors (β (SE) = 0.42 (0.16), p = 0.01), but not in patients where one or more risk factors were present (β (SE) = 0.06 (0.06), p = 0.35). The presence of major cardiovascular risk factors also impacted on the vaspin-angiopoetin-2 relationship, where vaspin levels were positively associated with those of angiopoetin-2 in patients with no major cardiovascular risk factors (β (SE) = 1.13 (0.05), p = 0.02). This study showed that vaspin concentrations were associated with reduced risk of plaque rupture, but only in those with low cardiovascular disease risk.

Impaired arterial function mediates cardiovascular events in non-RA persons. RA patients experience increased arterial stiffness. Several adipokines are associated with arterial stiffness in the general population. The third study investigated the relationships between nesfatin, visfatin and vaspin and measures of arterial function in 173 patients with RA. Arterial function measures of arterial stiffness, pressure pulsatility and wave reflection were assessed using applanation tonometry. In multivariate analysis, there were no significant associations between nesfatin or vaspin concentrations and any markers of arterial function. Visfatin concentrations were inversely associated with central augmentation pressure (β (SE) = -0.18 (0.06), p = 0.004), augmentation index (β (SE) = -0.15 (0.07), p = 0.02), reflection magnitude (β (SE) = -0.14 (-0.06), p = 0.04), central systolic blood pressure (β (SE) = -0.13 (0.06), p = 0.02) and central pulse pressure (β (SE) = -0.14 (0.06), p = 0.03). In stratified analysis, these associations remained significant only in older person and those with greater disease severity. The study showed that increased visfatin concentrations were associated with reduced wave reflection markers in RA patients who are older than 50 years of age and in patients with increased disease severity. Although this finding may seem paradoxical, recent evidence has shown decreased

wave reflection in older adults and those exposed to inflammation, in the presence of increased arterial stiffness. The reduced wave reflection increases the pulsatile energy that is absorbed in the periphery. Therefore, reduced wave reflection, especially in older adults is associated with increased risk of target organ damage. The results are in keeping with previous studies that suggest visfatin may be associated with adverse vascular remodelling.

RA patients experience an increased risk of developing left ventricular (LV) diastolic dysfunction and heart failure with a preserved ejection fraction. The role of adipokines as biomarkers for the development of heart failure is not well described. The final study investigated the association between nesfatin, visfatin and vaspin and diastolic function markers in 170 RA patients. Cardiac function was assessed by echocardiography. In multivariate regression analysis, visfatin concentrations were independently associated with E/e', an index of LV filling pressure (β (SE) = 0.21 (0.08), p = 0.008), and left atrial volume index (LAVI) (β (SE) = 0.17 (0.07), p = 0.02). In stratified analysis the association between visfatin and increased filling pressures remained significant in younger patients and those with lower disease severity. In stratified analysis, nesfatin concentrations were associated with reduced risk of LV concentric hypertrophy (relative wall thickness and LV mass index) in younger patients and in those with a shorter disease duration. Vaspin concentrations were associated with reduced LV relaxation (reduced lateral wall mitral annular velocity) in older patients. This study showed disparate associations between visfatin, nesfatin and vaspin concentrations and markers of adverse cardiac structure and function, and that these associations are impacted by age and disease severity.

In conclusion, the findings reported in this thesis contribute to the understanding of the roles of nesfatin, visfatin and vaspin as possible mediators of subclinical cardiovascular disease in RA patients. The findings suggest that nesfatin is associated with reduced atherosclerosis and increased plaque stability mediator levels and a decreased risk of adverse LV remodelling in RA. Vaspin concentrations are associated with reduced risk of plaque rupture and vessel stability, but only in those in the early stages of disease and with low cardiovascular disease risk. Visfatin relates to adverse cardio-metabolic risk in RA and is a likely biomarker for adverse vascular remodelling, plaque stability and increased LV filling pressures. Taken together, these adipokines may improve risk stratification for cardiovascular disease in RA patients. Publications and presentations arising from this thesis.