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Sitting time is negatively related to microvascular endothelium-dependent function in Rheumatoid Arthritis

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Abstract

Background: Sedentary behaviour is linked to increased cardiovascular disease risk in Rheumatoid Arthritis (RA), but the biological processes underlying this relationship are not understood. **Objectives:** To investigate the cross-sectional associations of habitual sedentary behaviour, with endothelial function in RA. **Methods**: Sixty-eight RA patients (Mage = 55±12 years) underwent Laser Doppler Imaging with iontophoresis, to assess microvascular endothelium-dependent (acetylcholine, ACh) and endothelium-independent (sodium nitroprusside, SNP) function. Large-vessel endothelium-dependent and endothelium-independent functions were measured via flow-mediated dilation (FMD) and glyceryl trinitrate dilation (GTN), respectively. Habitual sedentary behaviour (hours/week sitting) was self-reported (International Physical Activity Questionnaire). **Results:** Regressions revealed sitting time significantly negatively predicted microvascular endothelium-dependent function (ACh, $_{\text{unstandardized}}\beta = -3.25$, $p = .02$, 95% CI [– 6.07, – .42], $R^2 = 0.06$), but did not associate with other endothelial function outcomes (SNP, FMD, GTN). **Conclusion:** Habitual sitting time appears to be adversely linked to microvascular endothelium-dependent function among people living with RA.

Keywords: Sedentary behaviour; Rheumatoid arthritis; Endothelial function; Cardiovascular disease; Ultrasonography.

Introduction

Cardiovascular disease (CVD)[1] is the leading cause of death among people living with Rheumatoid Arthritis (RA) [1], with RA increasing CVD risk by ~50% compared to the general population [2]. High levels of sedentary behaviour (*waking behaviour ≤1.5 metabolic equivalents, whilst sitting/lying)* are linked to increased CVD risk in RA, independently of the benefits of physical activity [3]. Whilst the biological mechanisms underlying this adverse relationship are not yet known, recent experimental work suggests endothelial dysfunction may play an important role [4].

The endothelium maintains vascular homeostasis by regulating vascular tone and anti-atherosclerotic processes via the release of vasodilator molecules, such as nitric oxide (NO), prostacyclin (PGI2) and endothelium derived hyperpolarizing factor (EDHF) [5]. Several non-invasive assessments of NO-mediated vasodilation (i.e., endothelium–dependent function) can be conducted in the microvessels and large-vessels, and provide early indication of future CVD risk in the general population [6]. RA patients also have endothelial dysfunction which likely results from subtle interactions between inflammation and classical CVD risk factors [7], adversely affecting downstream endothelium-independent vasodilatory processes (i.e., smooth muscle cell integrity).[5] Indeed, RA patients exhibit poor microvascular perfusion in the coronary circulation, even when the larger epicardial arteries are clear, which suggests that different vascular beds are affected differently by RA-related factors [8]. However at present, it is not clear which factors affect the specific vascular outcomes in RA (i.e., endothelium-dependent vs. independent function in the small vs. large-vessels), and identification of such factors (e.g., the role of sedentary behaviour) is necessary to inform effective CVD prevention in this high-risk population.

To date, the majority of research investigating the implications of sedentary behaviour for endothelial function, has employed experimental designs to examine the effects of prolonged sitting (i.e., 3-5 hours uninterrupted sitting in a laboratory) on large-vessel endothelium-dependent function in healthy males [9]. Research investigating the impact of more habitual (daily) sedentariness on microvascular and large-vessel endothelial function is required to better evaluate its role in the development of CVD. This is important to investigate in RA specifically, as the mechanisms that drive vascular dysfunction may differ to those in healthy adults [10].

The aim of this study was therefore to examine the cross-sectional associations between habitual sedentary behaviour, with microvascular and large-vessel endothelium-dependent, and endothelium-independent function, in patients with RA. The data presented herein represents secondary analysis of previously published data, reporting associations between CVD risk and endothelial function in this cohort [11].

Materials and Methods

Ninety-eight RA patients were recruited from Rheumatology outpatient clinics at Russells Hall Hospital (Dudley Group NHS Foundation Trust). All patients recruited met the 1987 RA criteria of the American College of Rheumatology. Informed consent was obtained from all individual participants included in the study. Ethical approval was granted by the local National Health Service Research Ethics Committee (approval number:10/H1206/59).

[1] CVD, cardiovascular disease; RA, Rheumatoid Arthritis; NO, Nitric Oxide; DAS, Disease Activity Score; ESR, Erythrocyte Sedimentation Rate; HAQ, Health Assessment Questionnaire; ACh, Acetylcholine; SNP, sodium nitroprusside; FMD, Flow Mediated Dilation; GTN, Glyceryl-trinitrate.

Participants reported to a temperature-controlled vascular laboratory (22°C) to complete assessments, in a fasted state (12-hours) having refrained from exercise for 24hrs. *RA characteristics;* Disease activity was assessed using the Disease Activity Score in 28-joints (DAS28) and erythrocyte sedimentation rate (ESR). Disease severity was measured via the Stanford Health Assessment Questionnaire (HAQ). Use of vasoactive medication (i.e., anti-hypertensives, beta-blockers and/or calcium channel blockers) was self-reported and corroborated with medical notes.

Global (10-year) CVD risk; QRISK2 was used to indicate ten-year CVD risk. QRISK2 score was calculated using participants; age, gender, height, weight, blood pressure, cholesterol (total/HDL ratio), smoking status, diabetic status, presence of kidney disease and family history of heart disease [11].

Endothelial function; First, microvascular endothelial function was assessed non-invasively in the forearm, using Laser Doppler Imaging with iontophoresis of 1% Acetylcholine (ACh, endothelium-dependent function) and 1% sodium nitroprusside (SNP, endothelium-independent function), in 2.5ml solution containing 0.5% saline, according to previously established guidelines [12]. Following this, large vessel endothelium-dependent (flow mediated dilatation, FMD) and endothelium-independent (sublingual glyceryl-trinitrate, GTN) function, were measured using high-resolution Doppler Ultrasonography of the brachial artery [12]. Assessments of microvascular endothelial function were conducted first, as both FMD and GTN may affect blood flow in the forearm, and therefore iontophoresis measurements. Large vessel endothelium-independent function was assessed last, as administration of GTN causes systemic vasodilation, which would affect all preceding vascular tests.

Endothelial function was expressed as the percentage increase in perfusion or diameter from baseline. A single observer conducted all vascular assessments (AS), reporting intra-observer coefficients of variation of 6.5% (ACh), 5.9% (SNP), 10.7% (FMD) and 11.8% for (GTN). Data for ACh/SNP and GTN were not collected from 3 participants due to technical problems with equipment.

Sitting time; Habitual sitting-time was self-reported using the International Physical Activity Questionnaire (IPAQ). Participants reported their average time spent sitting on; 1) weekdays, and 2) weekend days (i.e., at home, whilst studying, leisure time), over the previous 7-days. Total weekly sitting time (hours/week) was computed; (weekday sitting time x 5) + (weekend day sitting time x 2).

Of the initial 98 participants recruited, 30 were excluded on the basis of missing IPAQ data (n = 27, missing data = 28%), or as extreme outliers (ACh/SNP, n = 3). Following these exclusions, missing data were < 5% for; QRISK2 = 2, DAS28 = 2, HAQ = 1, ACh/SNP = 3, GTN = 2). Missing values were therefore imputed to maximise statistical power (expectation maximisation method), retaining a final sample of n = 68 for statistical analyses. Participants in this final sample were not significantly different to those excluded (n = 30) for all targeted variables (Table 1).

Cross-sectional associations between habitual sitting time and endothelial function outcomes were examined via multiple regression analyses, in conjunction with bootstrapping. Bootstrap-generated 95% bias-corrected confidence intervals were constructed for 5000 samples [13], and analyses were adjusted for RA characteristics, global CVD risk, and vasoactive medication. Bootstrapping is a non-parametric resampling procedure reported to be superior to alternative tests with respect to Type 1 error rates and power (Table 1).[13] Analysis was performed using SPSS (version 24.0).

Results

Descriptive statistics are reported in Table 1. The sample was largely female, with moderate disease activity and moderate-to-severe disability. Regression analysis (Table 2) revealed habitual sitting time was significantly negatively related to microvascular endothelium-dependent function (ACh, $_{unstandardized}\beta = -3.25$, $p = .02$), but not microvascular endothelium-independent function (SNP, $_{unstandardized}\beta = -1.94$, $p = .07$). Sitting time accounted for 6% of the variance in microvascular endothelium-dependent function, with the total model explaining 18% of the variance in this outcome. Habitual sitting time did not significantly predict large-vessel endothelium-dependent vasodilation (FMD, $_{unstandardized}\beta = .01$, $p = .84$) or independent vasodilation (GTN, $_{unstandardized}\beta = -.06$, $p = .37$).

Discussion

This is the first study to reveal that higher self-reported sitting time is predictive of impaired microvascular endothelium-dependent function among people with RA. This cross-sectional association was observed after adjusting for global CVD risk, RA characteristics and vasoactive medication. Results provide new evidence to suggest "too much sitting" may be linked to poorer endothelial function, and contribute toward increased CVD risk in RA [1, 3].

A recent experimental study in healthy males reported significantly reduced hyperaemic response in the microvessels, but not the large-vessels of the brachial artery, following 6-hours of uninterrupted sitting [14]. Our results build on this work, to suggest that habitual sedentarity [i.e., repeated episodes of prolonged (uninterrupted) sitting], may lead to chronic impairments in peripheral microvascular endothelium-dependent function among people living with RA. In addition, these data strengthen the suggestion that the peripheral microvasculature may be more vulnerable to the adverse consequences of sedentary behaviour, relative to the large-vessels.

Reduced blood flow and associated shear rate are the mechanisms proposed to underlie the association between sitting and microvascular endothelium-dependent dysfunction. That is, in the absence of muscle activation (e.g., during low-energy sitting), peripheral vascular blood flow and shear rate are reduced [14], which in turn may lead to reduced NO bioavailability, and reduced vasodilatory function of the microvasculature [4, 9, 14]. Through such mechanisms, high levels of sitting time may perpetuate an already unfavourable CVD profile, and further contribute towards promoting vascular dysfunction and cardiovascular co-morbidity in RA. Indeed, vascular dysfunction in the peripheral circulation is reported to be a good predictor of long-term cardiovascular events in individuals with atherosclerosis, and healthy older participants [15, 16].

The impact of sitting on microvascular but not large-vessel endothelial function could be due to the microvessels comprising the largest proportion of the vasculature [17]. Thus, the microvasculature is likely to have greater exposure to damaging stimuli - e.g., the physiological processes associated with sedentary behaviour. As such, repeated episodes of sedentary behaviour may compromise the function of the microvessels, prior to (and to a greater extent than), any observed impairments in the large-vessels. Indeed, microvascular abnormalities can occur before, or alongside the development of CVD risk factors in healthy individuals, and among those with hypertension [18-20]. Coronary microvascular disease is also apparent in the absence of large-vessel disease in RA [8].

Our study did not demonstrate associations between habitual sitting time and endothelium-independent microvascular and large-vessel function in RA. This may suggest that the adverse effects of sitting relate primarily to the functionality of the endothelial cells

themselves (i.e., NO availability), rather than the integrity of the smooth muscle cells to relax [5]. However, this study reports secondary analysis of an existing data set, which may be underpowered to detect such associations. Indeed, standardised effect sizes were comparable for endothelium-dependent and endothelium–independent microvascular function outcomes (Table 2 legend). Thus, studies designed specifically to assess the role of sitting for endothelium-independent function in RA are required. Other limitations to this study include a cross-sectional design and a reliance on self-reported sedentary behaviour. Larger prospective and experimental studies utilising objective measurement devices are therefore required to confirm current findings. Insights regarding the specific mechanisms by which sitting time may lead to endothelial dysfunction in RA may be provided by experimental studies that compare this clinical population with healthy controls.

In conclusion, habitual self-reported sitting time appears to be associated with microvascular endothelium-dependent function in people with RA, but not large-vessel endothelial function. Results highlight the importance of experimental studies to confirm whether sedentary behaviour represents a modifiable risk factor for CVD in RA.

References

1. Kitas GD, Gabriel SE (2011). Cardiovascular disease in rheumatoid arthritis: state of the art and future perspectives. Ann Rheum Dis, 70:8-14.
2. Agca R, Heslinga SC, Rollefstad S et al. (2017). EULAR recommendations for cardiovascular disease risk management in patients with rheumatoid arthritis and other forms of inflammatory joint disorders: 2015/2016 update. Ann Rheum Dis, 76:17-28.
3. Fenton SAM, Veldhuijzen van Zanten JJCS, Kitas GD et al. (2017). Sedentary behaviour is associated with increased long-term cardiovascular risk in patients with rheumatoid arthritis independently of moderate-to-vigorous physical activity. BMC Musculoskelet Disord, 18:131.
4. Carter S, Hartman Y, Holder S (2017). Sedentary Behavior and Cardiovascular Disease Risk: Mediating Mechanisms. Exerc Sport Sci Rev, 45:80-86.
5. Sandoo A, Veldhuijzen van Zanten JJCS, Metsios GS et al. (2010). The endothelium and its role in regulating vascular tone. Open Cardiovasc Med, 4:302-312.
6. Lerman A, Zeiher AM (2005). Endothelial function: cardiac events. Circulation, 111: 363-368.
7. Sandoo A, Veldhuijzen van Zanten JJCS, Metsios GS et al. (2011). Vascular function and morphology in rheumatoid arthritis: a systematic review. Rheumatology (Oxford), 50:2125-2139.
8. Toutouzas K, Sfikakis PP, Karanasos A et al. (2013) Myocardial ischaemia without obstructive coronary artery disease in rheumatoid arthritis: hypothesis-generating insights from a cross-sectional study. Rheumatology (Oxford), 52:76-80.
9. Trinity JD (2017). Something is definitely better than nothing: simple strategies to prevent vascular dysfunction. Clin Sci (Lond), 13:1055-1058.
10. Grace MS, Climie RE, Dunstan DW (2017). Sedentary Behavior and Mechanisms of Cardiovascular Disease-Getting to the Heart of the Matter. Exerc Sport Sci Rev, 45:55-56.
11. Sandoo A, Kitas GD, Carroll D et al. (2012). The role of inflammation and cardiovascular disease risk on microvascular and macrovascular endothelial function in patients with rheumatoid arthritis: a cross-sectional and longitudinal study. Arthritis Res Ther, 14:R117.
12. Sandoo A, Kitas GD (2015). A methodological approach to non-invasive assessments of vascular function and morphology. J Vis Exp, 96:e52339.
13. Shrout PE, Bolger N (2002). Mediation in experimental and nonexperimental studies: new procedures and recommendations. Psychol Methods, 7:422-445.
14. Restaino RM, Holwerda SW, Credeur DP et al. (2015). Impact of prolonged sitting on lower and upper limb micro- and macrovascular dilator function. Exp Physiol, 100:829-838.

15. Gokce N, Keaney JF. Jr, Hunter LM et al. (2003). Predictive value of noninvasively determined endothelial dysfunction for long-term cardiovascular events in patients with peripheral vascular disease. J Am Coll Cardiol, 41:1769-1775.
16. Schachinger V, Britten MB, Zeiher AM (2000). Prognostic impact of coronary vasodilator dysfunction on adverse long-term outcome of coronary heart disease. Circulation, 101:1899-1906.
17. Krentz AJ, Clough G, Byrne CD (2009). Vascular disease in the metabolic syndrome: do we need to target the microcirculation to treat large vessel disease? J Vasc Res, 46:515-526.
18. McVeigh GE, Bratteli CW, Morgan DJ et al. (1999). Age-related abnormalities in arterial compliance identified by pressure pulse contour analysis: aging and arterial compliance. Hypertension, 33:1392-1398.
19. Giannattasio C, Cattaneo BM, Mangoni AA et al. (1995). Cardiac and vascular structural changes in normotensive subjects with parental hypertension. J Hypertens, 13:259-264.
20. Longhini CS, Scorzoni D, Bazzanini F et al. (1997). The structural arteriolar changes in diabetes mellitus and essential hypertension. The relative contribution of ageing and high blood pressure. Eur Heart J, 18:1135-1140.

Table 1. Descriptive statistics

	Mean ± SD n = 68	**Range (min – max)**
Age (years)	55 ± 12	24 – 77
Gender (% female)	74%	
Height (cm)	164.0 ± 9.3	142.0 – 190.0
Weight (kg)	80.8 ± 18.2	48.7 – 122.9
RA characteristics		
Disease activity (DAS28)	3.56 ± 1.37	0.14 – 7.00
¶Disease severity (HAQ)	1.59 ± 0.88	0 – 3
Vasoactive medication (% yes)	47%	
CVD risk factors		
Total cholesterol (mmol/L)	4.9 ± 1.0	3.0 – 7.1
HDL cholesterol (mmol/L)	1.4 ± .35	0.8 – 2.4
Systolic blood pressure (mmHg)	130 ± 17	93 – 165
Diastolic blood pressire (mmHg)	80 ± 10	58 – 105
Body-mass-index (kg/m^2)	30.2 ± 6.4	20.3 – 45.9
Smoker (% current smokers)	19%	
Diabetes (% yes)	6%	
Kidney Disease (% yes)	2%	
Family history of heart disease (% yes)	47%	
¶QRISK2 (%)	18.4 ± 14.2	0 – 66
Endothelial function		
Microvascular function		
¶Endothelium-dependent (ACh)	316 ± 212	− 3 – 908
¶Endothelium-independent (SNP)	302 ± 180	− 4 – 832
Large-vessel function		
Endothelium-dependent (FMD)	9.4 ± 5.9	− 1.4 – 23.0
Endothelium-independent (GTN)	22.9 ± 8.5	4.8 – 44.4
Sitting time		
Weekday (hours)	5.5 ± 2.8	1.0 – 13.0
Weekend day (hours)	5.8 ± 2.6	1.5 – 13.0
Total weekly (hours)	38.9 ± 18.3	10.5 – 91.0

Note: ¶ = Non-normally distributed data (Kolmogorov-Smirnov p <.05). Log transformations did not reduce skewness in the case of HAQ. Consequently, non-parametric tests (i.e., Mann-Whitney (M-W) U, bootstrapping) were used in subsequent analyses that included non-normally distributed data.

Data is presented following imputation of missing values for the final sample of n = 68 participants included in regression analyses. Participants included were not significantly different from those excluded for; age (t (92) = −1.20), gender (χ^2 (1) = .10), all endothelial function outcomes (ACh (M-W U = −1.40); SNP (M-W U = −.76); FMD (t (90) = 1.11); GTN (t (89) = .35), QRISK2 (M-W U = .25), RA characteristics (DAS28 (t (90) = .13); HAQ (M-W U = 1.76)), vasoactive medication (χ^2 (1) = .31) [all p = >.08]

Table 2. Results of regression analyses examining associations between self-reported sitting time and endothelial function outcomes.

	ACh				SNP				FMD				GTN			
	β	*p*	95% CI [upper, lower]	R^2	β	*p*	95% CI [upper, lower]	R^2	β	*p*	95% CI [upper, lower]	R^2	β	*p*	95% CI [upper, lower]	R^2
Model 1																
Global CVD risk (Q-risk2)	– 4.81	.02*	– 8.62, – 1.58	.10	– 2.03	.28	– 5.83, 1.16	.01	– .11	.13	– .24, .01	.05	– .25	.00**	– .36, – .14	.24
Disease activity (DAS28)	20.75	.26	– 13.67, 55.50	.02	7.74	.68	– 25.45, 48.00	.01	.44	.52	– .91, 1.61	.01	– .25	.79	– 2.13, 1.38	.00
Disease severity (HAQ)	– 1.94	.95	– 74.37, 58.29	.00	– 57.38	.08	– 119.42, – 1.46	.05	.13	.90	– 1.72, 2.21	.00	1.50	.25	– .94, 4.52	.01
Vasoactive medication	– 12.68	.79	– 111.69, 87.89	.00	– 40.96	.32	– 117.86, 37.55	.01	– 1.30	.30	– 3.49, .93	.01	3.83	.04*	.56, 7.16	.05
Total Model 1		.09		.12		.23		.08		.28		.07		.00**		.30
Model 2																
Sitting time (hours/week)	– 3.25	.02*	– 6.07, – .42	.06	– 1.94	.07	– 4.32, –.14	.03	.01	.84	– .08, .10	.00	– .06	.37	– .17, .06	.01
Total Model 2		.03*		.18		.14		.11		.84		.07		.32		.31

Note: RA patients were recruited from Russells Hall Hospital between September 2008 and September 2010

β = bootstrapped path coefficients (unstandardized), * p <.05, ** p <.01. The 95% bootstrap bias corrected confidence intervals are reported (95% CI [upper, lower]). R^2 = variance in endothelial function explained by predictor variable. For *Total Model 1*, p = significance of model. For *Total Model 2*, p = significance of R^2 change.

Model 1 = relationships between global CVD risk, RA characteristics, and vasoactive medication with endothelial function.
Model 2 = associations between total weekly sitting time and endothelial function adjusting for factors in Model 1. Relationships reported in Model 2 were unchanged where non-bootstrapped regressions were computed. Standardised (non-bootstrapped) coefficients for the relationships between sitting time and endothelial function outcomes were; ACh, β = − .28, p = .03; SNP, β = − .20, p = .14; FMD, β = .03, p = .84; GTN, β = − .12, p = .32

CVD = Cardiovascular Disease; DAS28 = Disease Activity Score 28; HAQ = Health Assessment Questionnaire; ACh = Acetylcholine; SNP = Sodium Nitroprusside; FMD = Flow Mediated Dilation; GTN = Glyceryl Trinitrate.