Red Cell Distribution Width and Carotid Atherosclerosis Progression. The Tromsø Study.

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Running title: RDW and atherosclerosis

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Word count abstract: 228

Word count text including references: 4746

Number of Tables: 3

Number of Figures: 2

Number of references: 34

Extra table

What is known on this topic	What this paper adds
- RDW is associated with cardiovascular morbidity - RDW is associated with cardiovascular mortality and all-cause mortality - Recent studies have suggested a relationship between RDW and prevalence of atherosclerosis	- RDW is associated with prevalence of carotid atherosclerosis in the general population - RDW is associated with carotid plaque growth in the general population

Funding

K.G. Jebsen- Thrombosis Research and Expertise Centre (TREC) is supported by an independent grant from the K.G. Jebsen Foundation. SKB and JBH have received research grants from the Northern Norway Regional Health Authority (URL: http://www.helse-nord.no/). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Summary

Red cell distribution width (RDW), a measure of the size variability of circulating erythrocytes, is associated with cardiovascular morbidity and mortality. We aimed to investigate whether RDW was associated with progression of atherosclerotic plaques in subjects recruited from the general population.

Baseline characteristics, including RDW, were collected from 4677 participants in the fourth survey of the Tromsø Study conducted in 1994/95. Prevalence of carotid plaques and total plaque area (TPA) were assessed by ultrasonographic imaging at baseline and after 7 years of follow-up. Generalized linear models were used to analyse change in TPA across tertiles of RDW.

Change in TPA was significantly higher across tertiles of RDW in crude analysis and in multivariable analysis adjusted for cardiovascular risk factors. The mean change in TPA increased from 5.6mm^2 (4.9-6.4) in tertile 1 (RDW≤12.6%) to 6.7mm^2 (5.9-7.6) in tertile 3 (RDW≥13.3) in multivariable analysis adjusted for body mass index, total cholesterol, HDL cholesterol, systolic blood pressure, self-reported diabetes, smoking status, platelet count, white blood cell count, and hs-CRP levels (p for trend 0.003). A 1% increase in RDW was associated with 0.6 mm^2 (0.1-1.2) increase in TPA in multivariable analysis (p=0.03).

RDW was associated with progression of atherosclerosis after adjustments for traditional atherosclerotic risk factors. Our findings suggest that the link between RDW and cardiovascular morbidity and mortality may be explained by atherosclerosis.

Keywords: atherosclerosis, cardiovascular disease, cohort studies, epidemiology, erythrocyte indices

Introduction

Red blood cell distribution width (RDW) is an easy accessible and inexpensive measure of the size variability of circulating erythrocytes. RDW gives the coefficient of variation of the red blood cell volume in percentage, and is calculated by most automated blood cell counters. Traditionally, RDW together with mean corpuscular volume (MCV) is used in the differential diagnosis of anaemia. Iron deficiency anaemia is normally characterized by high RDW and low MCV [1], whereas both RDW and MCV are high in B12 and folic acid deficiencies. An increased RDW may also result from premature release of immature red blood cells into the blood stream, hemoglobinopathies or other haematological diseases [2-4].

Recent studies have reported that high values of RDW are associated with increased risk of cardiovascular diseases [5-9]. Moreover, RDW is associated with all-cause mortality in patients with heart failure [10-12], in male patients referred for coronary angiography [13], and in patients undergoing percutaneous coronary intervention [14]. The underlying mechanism for the association between RDW and cardiovascular disease and mortality is unknown.

Atherosclerosis is the principal cause of myocardial infarction, ischemic stroke and peripheral artery disease [15-17], and ischemic heart disease due to atherosclerosis is the leading cause of death worldwide [18]. The relationship between RDW and atherosclerosis has been scarcely investigated. In a cross-sectional study of 156 hypertensive patients, high RDW was associated with increased prevalence of carotid atherosclerosis [19]. In a cross-sectional study with almost 7000 participants, RDW was associated with higher prevalence of peripheral artery disease assessed by the ankle brachial index [7].

The underlying mechanism for the observed association between RDW and future risk of morbidity and mortality from cardiovascular diseases remains unclear. To our knowledge, the association between RDW and future progression of atherosclerosis has previously not

been addressed. Therefore, we set out to investigate whether high RDW was associated with carotid plaque progression in a cohort recruited from a general population.

Materials and Methods

Study population

In 1994-95 all inhabitants above 24 years in the municipality of Tromsø, Norway, were invited to the fourth survey of the Tromsø study. The participation rate was 77% with 27158 people attending the first visit [20]. In addition, participants aged 55-74 years as well as 5-10% samples in other 5-year age groups (25-54 years and 75-85 years) were offered a more extensive examination which included ultrasonography of their right carotid artery. In total, 7965 attended, and 6727 participated in the carotid ultrasound scanning. In 2001-02, all subjects who participated in the carotid ultrasound scanning and were still alive and inhabitants of Tromsø, were invited to the fifth survey of the Tromsø study. A total of 532 died and 271 moved between baseline and follow-up. Of the remaining, 4968 attended the fifth survey and 4858 were re-scanned with carotid ultrasound. After excluding subjects with missing measurements of RDW (n=105) or total plaque area (n=48), 4674 subjects were included in the present study. The study was approved by the regional ethical committee, and informed written consent was obtained from all participants.

Baseline measurements

Baseline measurements were collected through blood samples and physical examinations. Blood was drawn from an antecubital vein into vacutainer tubes containing EDTA as an anticoagulant (K3-EDTA 40 μL, 0.37 mol/L per tube). For measurement of blood cell counts, 5 ml of blood was drawn, and analysed within 12 hours in an automated blood cell counter (Coulter Counter; Coulter Electronics, Luton, UK). RDW was calculated as the standard deviation of MCV divided by the mean of MCV and multiplied by 100 to give the percentage. The analytic variation coefficient of RDW was less than 3%.

For lipid and high sensitivity C-reactive protein (hs-CRP) measurements, serum was prepared from non-fasting blood samples by centrifugation after one hour respite at room temperature. Triglycerides, total cholesterol, high density lipoprotein (HDL) cholesterol and hs-CRP were measured using commercial kits as previously described [21, 22]. The blood samples were analysed at the Department of Clinical Chemistry, University Hospital of North Norway. Blood pressure was recorded three times at one-minute intervals after two minutes of seated resting with the use of an automatic device (Dinamap Vital Signs Monitor 1846 Criticon), and the mean of the last two recordings was used in this report. Height and weight were measured in participants wearing light clothing and no footwear. Body mass index (BMI) was calculated as weight in kilograms, divided by the square of height in meters (kg*m-2). Information on smoking habits, alcohol consumption, dietary habits, diabetes, family history of cardiovascular diseases and concurrent diseases was obtained from self-administered questionnaires. The study participants' kidney function was estimated by calculating their glomerular filtration rate (GFR, mL/min/1.73 m^2) based on their creatinine clearance using the Modification of Diet in Renal Disease (MDRD) formula [23]. The subjects were staged as follows: Stage 1: GFR≥90, stage 2: GFR 60-89, stage 3: GFR 30-59, stage 4: GFR 15-29, stage 5 GFR<15. The World Health Organization's definition was used to identify subjects with anemia [24].

Ultrasound examination

High-resolution B-mode and colour Doppler/pulsed-wave Doppler ultrasonography of the right carotid artery was performed, with the participants randomly distributed among the sonographers [25]. An Acuson Xp10 128 ART ultrasound scanner equipped with a linear array 5-7 MHz transducer was used for the examination.

Plaques were investigated for in the near and far walls of the right common carotid, the bifurcation, and the internal carotid artery (6 locations). A plaque was defined as localized protrusions of more than 50% compared to adjacent intima-media thickness (IMT). The examinations and measurements of all plaques were recorded on videotapes. Subsequently, the stored B-mode images were digitized with the use of meteor II/Matrox Intellicam, a commercially available video grabber card. Plaque area was assessed with Adobe Photoshop image-processing program (version 7.0.1), by tracing the plaque perimeter with a cursor [26]. The outline of each plaque was marked manually on still images, with calculation of plaque area. In subjects with more than one plaque, TPA was calculated as the sum of all plaque areas. Plaque progression was defined as the difference in TPA between plaque measurements in the 5th and 4th survey of the Tromsø study. Reproducibility of the ultrasound examinations was acceptable [27].

Statistical analysis

Statistical analyses were performed using STATA version 13.0 (Stata corporation, College station, TX, USA). Categorical data were presented as numbers or percentages while continuous variables were presented as means with standard deviations (SD). Baseline characteristics across categories of RDW were adjusted for age by analysis of variance (ANOVA).

TPA was square root transformed to approximate normal distribution. The change in TPA across tertiles (T) of RDW (T1: ≤12.6%; T2: 12.7-13.2%; T3: ≥13.3%), and per 1% increase in RDW, was evaluated with generalized linear regression, and adjustments for potential confounders were conducted using different models. Variables previously shown to be independently associated with plaque growth were included in the adjustment models. The multivariable adjustment model (model 3) included age, sex, systolic blood pressure, HDL

cholesterol, total cholesterol, current smoking, self-reported diabetes, BMI, white blood cell count, platelet count and hs-CRP. Model 4 included the same variables as model 3, as well as baseline total plaque area. The number of subjects included in the different adjustment models varied slightly due to missing data for some co-variates (in total <1 % missing). To ensure that the results were not confounded by renal disease, history of cardiovascular disease or anemia, we adjusted for stages of kidney function in the multivariable models, and re-ran all analyses after exclusion of subjects with cardiovascular disease (myocardial infarction and stroke) and anemia, respectively. Statistical interactions between RDW and age and sex were tested, and no significant interactions were found.

Results

Characteristics of study participants at baseline (Tromsø 4) and follow-up (Tromsø 5) are shown in Table 1. Average total cholesterol, HDL cholesterol and white blood cell count decreased in the study population between the Tromsø 4 and Tromsø 5 survey, while BMI and the proportion with self-reported diabetes increased. During the time period between Tromsø 4 (1994-95) and Tromsø 5 (2001-02), novel plaque formation occurred in 1017 (40.2%) of 2528 subjects with no plaque at baseline. In the lowest RDW category, 33.2% of the subjects developed plaque, while 50.5% developed plaque in the category with the highest RDW values. The prevalence of plaques among study participants increased from 46.0% in 1994-95 to 62.3% in 2001-02, whereas average total plaque area in all subjects increased from 8.98 ± 15.34 mm^2 to 15.22 ± 19.68 mm^2 (Table 1).

Age-adjusted baseline characteristics across tertiles of RDW are shown in table 2. Age, the proportion of smokers, hs-CRP, white blood cell count and platelet counts increased across tertiles of RDW, whereas haemoglobin levels and the proportion of subjects with diabetes decreased. The proportion of subjects with plaque at baseline increased from 38.2% in tertile 1 to 51.3% in tertile 3 of RDW (age-adjusted p for trend=0.001), while TPA increased from 6.58 mm^2 to 10.85 mm^2 across tertiles of RDW (age- and sex-adjusted p for trend=0.002) (Table 2).

Higher tertiles of RDW was associated with increased progression of TPA in both crude and multivariable analysis (Table 3). The mean change in TPA increased from 5.6 mm^2 (4.9-6.4) in tertile 1 to 6.7 mm^2 (5.9-7.6) in tertile 3 after adjustment for traditional atherosclerotic risk factors (model 3) (p for trend = 0.003). The association remained significant even after adjustment for TPA at baseline. Inclusion of kidney function in adjustment model 3 did not alter the results (data not shown).

When RDW was analysed as a continuous variable, 1% increase in RDW yielded 0.9 mm^2 (0.4-1.4) increase in TPA after adjustment for age and sex (p=0.001), and 0.6 mm^2 (0.1-1.2) after additional adjustment for traditional atherosclerotic risk factors (p=0.03) (table 3). Exclusion of subjects who reported a history of myocardial infarction or stroke prior to the first examination (n=304) did not alter the results. Neither did the exclusions of subjects with anemia (n=100) (data not shown).

Discussion

Our study is, to the best of our knowledge, the first population-based cohort to explore the relationship between RDW and the burden of atherosclerosis. RDW was associated with prevalence of plaques and TPA at baseline. Furthermore, RDW at baseline predicted future progression of TPA in both categorized and linear models adjusted for traditional atherosclerotic risk factors. Our findings suggest that atherosclerosis may explain the observed relation between RDW and cardiovascular morbidity and mortality.

Only a few previous studies have investigated the association between RDW and atherosclerosis. In a cross-sectional study of 156 middle-aged and elderly hypertensive patients [19], the proportion of subjects with carotid atherosclerosis increased across increasing categories of RDW. In the National Health and Nutrition Examination Survey (NHANES) [7], a cross-sectional study of almost 7000 subjects, high RDW (≥13.1%) was associated with increased prevalence of peripheral artery disease after adjustment for cardiovascular risk factors, history of cardiovascular diseases and iron status. In our large cohort of subjects recruited from a general population, we showed that RDW was associated with plaque burden at baseline and future plaque progression.

Growing evidence support an association between RDW and cardiovascular morbidity and mortality [5, 6, 28, 29]. In a cohort of 225,006 patients >40 years of age, followed for 5 years, the risk of major cardiovascular events was moderately increased, and the risk of total mortality was 4.6-fold higher in men, and 3.3-fold higher in women with RDW above 17% compared to RDW equal to or below 13% [30]. In the European Prospective Investigation into Cancer and Nutrition (EPIC) - Norfolk cohort, RDW in the upper quartile (>13.8%) was associated with 40% increased risk of heart failure compared to those with RDW in the lowest quartile [31], and the association was independent of traditional cardiovascular risk factors, inflammation and iron metabolism. In the NHANES study, RDW was a powerful

predictor of future risk of coronary heart disease. The pathophysiological mechanism behind these associations remains unclear. Rupture of an atherosclerotic plaque with subsequent thrombus formation is the principal cause of acute cardiovascular events [32]. Our findings suggest that formation and growth of atherosclerosis may play a role for the association between RDW and cardiovascular morbidity and mortality.

RDW is strongly related to inflammation, oxidative stress and other systemic factors that in different ways interfere with the erythropoiesis. Previous studies have reported that RDW is associated with inflammation (assessed by CRP), erythrocyte sedimentation rate, and various inflammatory cytokines [28, 33]. On the other hand, studies have shown that RDW predicted cardiovascular outcomes and mortality even after adjustment for CRP [6]. Accordingly, we found that RDW predicted carotid plaque progression even in analysis adjusted for hs-CRP. These findings suggest that mechanisms other than inflammation may be involved in initiation and progression of atherosclerosis by high RDW.

The size distribution of erythrocytes may influence their ability to be incorporated into atherosclerotic plaques. The erythrocyte membrane contains large amounts of free cholesterol [34], and accumulation of erythrocytes within the atheromatous plaque may promote plaque growth and instability [35]. Unstable plaques rich in cholesterol are vulnerable to rupture and thereby prone to cause acute atherothrombotic events.

A major strength of our study is the prospective population-based design with subjects recruited from the general population. The attendance rate was high, and few participants were lost to the follow-up examination. In total, 88% of those invited attended the Tromsø 4 survey, and 86% attended Tromsø 5. All blood samples were analysed in the same laboratory, and we were able to adjust for many potential confounders. Some limitations merit consideration. There were no repeated measurements of RDW, which gives rise to potential random measurement error, and we could not determine whether changes in RDW over time

would influence atherosclerosis. As in all cohort studies, residual confounding cannot be completely ruled out, and we were not able to take into account any potential medication use by the study participants. Although carotid artery atherosclerosis is considered to be a bilaterally symmetrical disease, cases may have been lost because only the right carotid artery was examined by ultrasonography [36]. The ultrasonography was carried out by three different examiners which increase the chance of misclassification. When measuring progression of carotid plaques, there is a risk of random measurement errors accumulating from baseline and follow-up which attenuate the differences we are able to detect. Nevertheless, our findings indicate that atherosclerosis may be an important mediator for the observed association between RDW and cardiovascular morbidity and mortality in the population.

In conclusion, we found that RDW was associated with prevalence and burden of carotid plaques at baseline, and with future plaque progression independent of traditional atherosclerotic risk factors including hs-CRP. Our findings suggest that RDW confers atherosclerosis progression, independent of low-grade inflammation, which may explain the observed relationship between RDW and cardiovascular morbidity and mortality.

Author contributions

J. Lappegård – analysed the data and drafted the manuscript

T.S. Ellingsen – interpreted the results and revised the manuscript

T. Skjelbakken – interpreted the results and revised the manuscript

J. Brox - interpreted the results and revised the manuscript

E.B. Mathiesen - interpreted the results and revised the manuscript

S.H. Johnsen – contributed with data collection

S.K. Brækkan – designed the study, interpreted the results, and revised manuscript

J.B. Hansen - designed the study, interpreted the results, and revised manuscript

Acknowledgements

The authors thank participants of The Tromsø Study for their important contributions.

Disclosure

No conflict of interest

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Table legends

Table 1 Characteristics of study participants

Characteristics of study participants at baseline and follow-up (n=4674). The values are reported as means ± standard deviations, or numbers with percentages in brackets.

Table 2 Age-adjusted baseline characteristics

Age-adjusted baseline characteristics across tertiles of red cell distribution width (RDW).

Table 3 Mean change in carotid plaque area

Mean change in carotid plaque area (mm^2) during follow-up across tertiles of red cell distribution width (RDW) and per 1% increase in RDW.

Figure legends

Figure 1

Mean total plaque area (TPA) at baseline, adjusted for age, across tertiles of Red cell distribution width (RDW). Black line shows 95% Confidence Intervals.

Figure 2

Mean change in total plaque area (TPA) across tertiles of Red cell distribution width (RDW). Values are adjusted for age, sex, total cholesterol, HDL cholesterol, systolic blood pressure, self-reported diabetes, current smoking, platelet count, white blood cell count, BMI and hs-CRP. Black line shows 95% Confidence Intervals.

Table 1 Characteristics of study participants

	Tromsø 4 (1994-1995)	**Tromsø 5 (2001-2002)**
Age	59.4 ± 9.6	-
Sex (% men)	48.3	-
Current smoker	1406 (30.1%)	1168 (25.0%)
Body mass index (kg/m^2)	26.0 ± 3.7	26.9 ± 4.1
RDW (% variation)	13.0 ± 0.84	-
Systolic blood pressure (mmHg)	144 ± 22	144 ± 22
Diastolic blood pressure (mmHg)	83 ± 13	82 ± 13
Total cholesterol ($mmol\ L^{-1}$)	6.74 ± 1.26	6.31 ± 1.19
HDL cholesterol ($mmol\ L^{-1}$)	1.55 ± 0.43	1.48 ± 0.41
White blood cells ($10^9\ L^{-1}$)	6.87 ± 1.85	6.36 ± 1.85
CRP (mg/L)	2.42 ± 6.35	3.34 ± 7.15
Thrombocytes ($10^9\ L^{-1}$)	247.8 ± 56.5	247.3 ± 58.8
Diabetes Mellitus	109 (2.33%)	219 (4.68%)
History of cardiovascular disease	304 (6.5%)	608 (13%)
Number (%) with plaques	2149 (46.0%)	2915 (62.3%)
1 plaque	1349 (28.8%)	1316 (28.1%)
2 plaques	574 (12.3%)	947 (20.3%)
3 plaques or more	226 (4.8%)	652 (13.9%)
Plaque area (mm^2)	8.98 ± 15.34	15.22 ± 19.68

Table 2 Age-adjusted baseline characteristics

	T1 (n=1560)	*T2 (n=1721)*	*T3 (n=1393)*
RDW (range)	11.0-12.6	12.7-13.2	13.3-25.2
Age (years)	57.2 ± 10.5	59.8 ± 8.8	61.5 ± 8.7
Sex (% men)	42.6	50.5	52.1
Current smoker (%)	20.4	29.9	41.4
Body mass index (kg/m^2)	26.0 ± 3.7	26.1 ± 3.7	25.9 ± 3.8
Systolic blood pressure (mm Hg)	143 ± 21	144 ± 22	144 ± 22
Diastolic blood pressure (mm Hg)	82 ± 12	83 ± 13	84 ± 13
Total cholesterol ($mmol\ L^{-1}$)	6.67 ± 1.24	6.77 ± 1.25	6.78 ± 1.27
HDL cholesterol ($mmol\ L^{-1}$)	1.54 ± 0.42	1.53 ± 0.42	1.58 ± 0.45
Diabetes mellitus	2.6	2.6	1.7
Red blood cells ($10^{12}\ L^{-1}$)	4.62 ± 0.37	4.68 ± 0.38	4.66 ± 0.42
Haemoglobin ($g\ dL^{-1}$)	14.2 ± 1.0	14.3 ± 1.0	14.0 ± 1.2
Thrombocytes ($10^9\ L^{-1}$)	245 ± 51	246 ± 52	253 ± 67
White blood cells ($10^9\ L^{-1}$)	6.61 ± 1.74	6.83 ± 1.73	7.20 ± 2.07
hs-CRP ($mg\ L^{-1}$)	2.07 ± 5.77	2.23 ± 5.37	3.05 ± 7.87
% with plaques	38.2	48.6	51.3
1 plaque	25.3	30.9	30.3
2 plaques	9.9	12.4	14.8
3 plaques or more	3.1	5.9	6.2
Plaque area (mm^2)	7.57	9.46	10.21

Continuous variables are reported as mean ± standard deviation, and categorical values as percentages.

Table 3 Mean change in carotid plaque area

	T1 (n=1560)	*T2 (n=1721)*	*T3 (n=1393)*	*P(trend)**	*Per 1 % RDW*	*P value*
Model 1	4.7 (3.9-5.4)	6.4 (5.7-7.1)	7.5 (6.7-8.3)	<0.001	1.4 (0.9-1.9)	<0.001
Model 2	5.3 (4.5-6.0)	6.3 (5.5-7.0)	7.0 (6.2-7.8)	<0.001	0.9 (0.4-1.4)	0.001
Model 3	5.6 (4.9-6.4)	6.4 (5.7-7.1)	6.7 (5.9-7.6)	0.003	0.6 (0.1-1.2)	0.03
Model 4	5.4 (4.7-6.2)	6.5 (5.8-7.2)	6.9 (6.1-7.7)	0.011	0.8 (0.3-1.3)	0.004

Values are means (95% confidence intervals).

*Square root transformed values of TPA were used to assess the P(trend) values

Model 1: Unadjusted

Model 2: Adjusted for age and sex

Model 3: adjusted for age, sex, total cholesterol, HDL cholesterol, systolic blood pressure, self-reported diabetes, current smoking, platelet count, white blood cell count, BMI and hs-CRP

Model 4: Model 3 + baseline plaque area

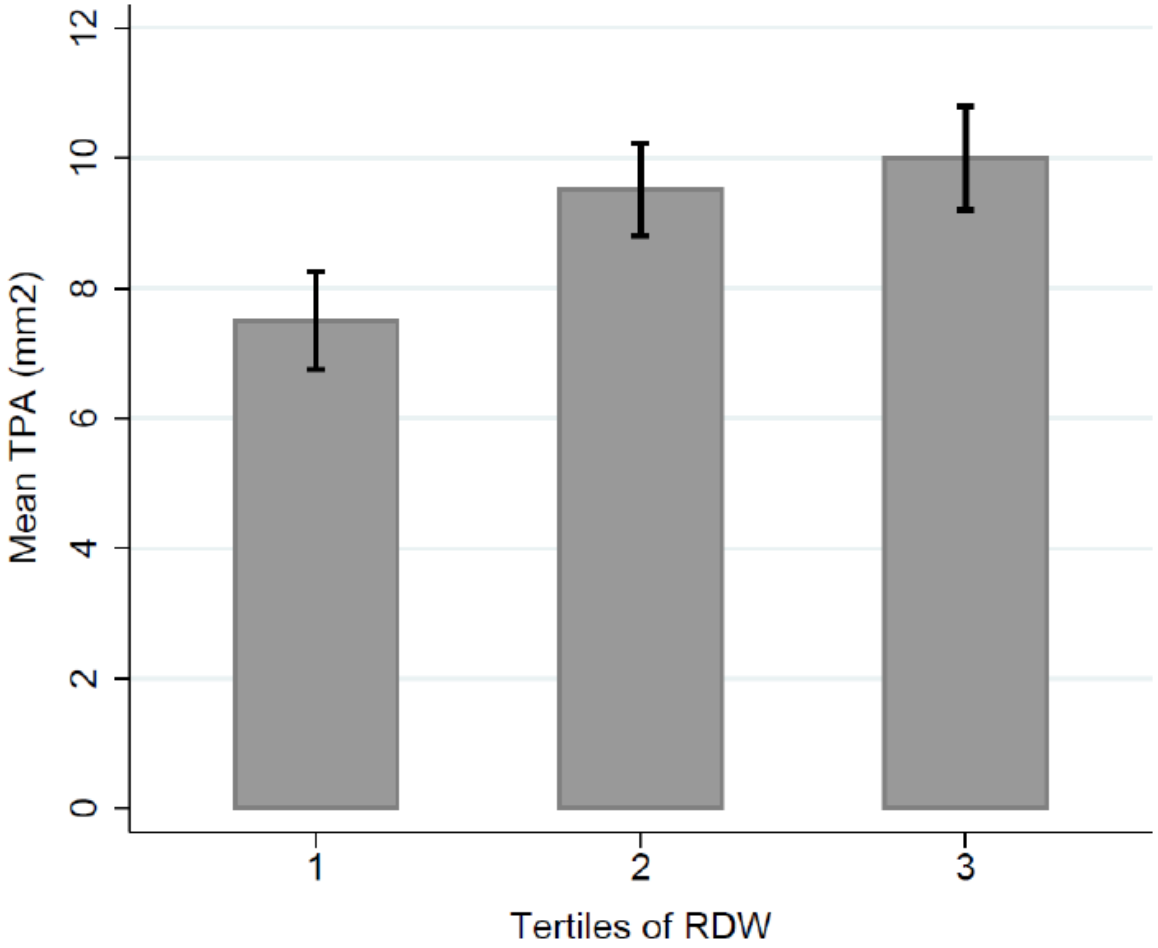

Figure 1

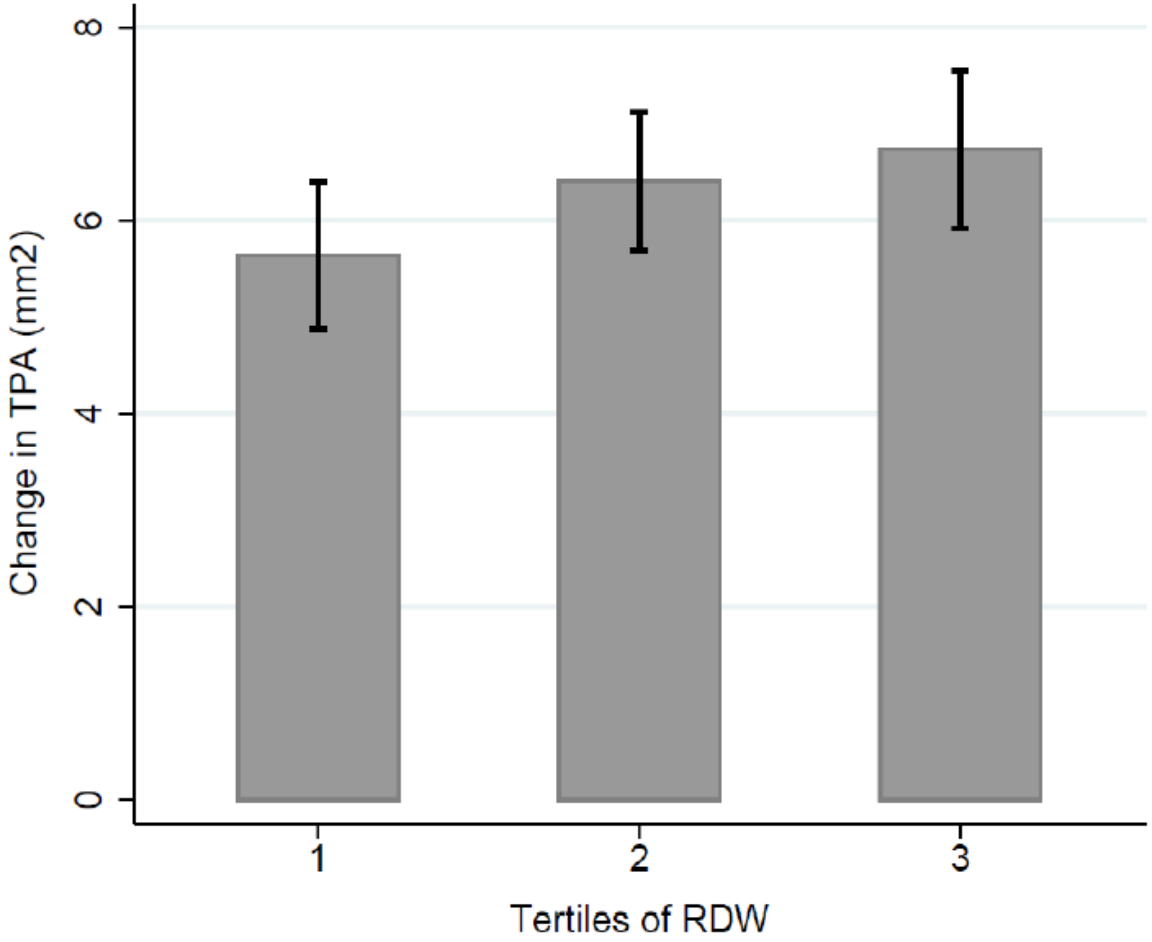

Figure 2