The relationship of body fatness and body fat distribution with microvascular recruitment: The Amsterdam Growth and Health Longitudinal Study

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Abstract

Introduction Microvascular function has been proposed to link body fatness to CVD and DM2. Current knowledge of these relationships is mainly based on studies in selected populations of extreme phenotypes. Whether these findings can be translated to the general population remains to be investigated.
Aim: To assess the relationship of body fatness and body fat distribution with microvascular function in a healthy population based cohort.
Methods: Body fatness parameters were obtained by anthropometry and whole-body dual-X-ray absorptiometry (DEXA) in 2000 and 2006. Microvascular recruitment (i.e., absolute increase in perfused capillaries after arterial occlusion, using nailfold capillaroscopy) was measured in 2006. Linear regression analysis was used to examine the relationship of (changes in) body fatness and body fat distribution with microvascular recruitment.
Results: Data were available for 259 participants (116 men). Capillary density

was higher in women than in men (difference 7.3/mm2; $p < 0.05$). In the total population, the relationship between total body fatness and microvascular recruitment was positive ($b = 0.43$; $p = 0.002$), whereas a central pattern of fat distribution (trunk-over-total fatness) showed a negative relationship ($b =)26.2$; $p = 0.032$) with microvascular recruitment. However, no association remained apparent after adjustment for gender. In addition, there was no relationship between 6-year changes in body fatness or fat distribution and microvascular recruitment.
Conclusion: Women show higher capillary recruitment values than men. This study does not support a linear relationship between microvascular function and body fatness or body fat distribution within a population-based normal range.

Key words: obesity, body fat distribution, microvascular recruitment, epidemiology

Abbreviations used: AGHLS, Amsterdam Growth and Health Longitudinal Study; BMI, Body Mass Index; CVD, CardioVascular Disease; DEXA, Dual energy X-ray Absorptiometry; DM2, Type 2 Diabetes Mellitus; HDLc, High Density Lipoprotein cholesterol; ICC, Intra-class Correlation Coefficient; NCEP, National Cholesterol Education Program.

Introduction

Epidemiological studies of the last decades report a rapid increase in prevalence of obesity and obesity-related morbidity and mortality[6,16,17]. Obesity, as a risk factor alone, or as part of the metabolic syndrome cluster, is related to CVD, DM2, and related disorders[186,187]. Although this relationship is well established, the underlying mechanisms are far from clear[30,188]. Microvascular function has been proposed to explain at least part of the relationship between body fatness and CVD risk, as several studies observed perturbed microvascular structure and function in obesity[1,2,4,6,7,189]. Not only total body fatness, but also a central pattern of fat distribution (i.e., trunk / total fatness ratio), is thought to affect the vasculature[20–22]. However, whether body fatness and body fat distribution have different or complementary effects on microvascular function remains unclear[22,183,187,188,190].

Current knowledge on the relationship of body fatness and body fat distribution with microvascular recruitment is mainly based on studies using small populations, populations with extreme degrees of obesity, or animal studies[4,6,189]. Whether the relationships found in these studies can be translated to the general population, in which extremes make up only a very small proportion of the total, is yet to be tested. Only two studies investigated larger populations[29,30,] but these were also not truly population-based, as some

degree of subject selection could not be avoided. In the current observational study, performed in an apparently healthy, population-based cohort, we examined the potential existence of a linear relationship of body fatness and body fat distribution with microvascular recruitment.

Methods

Study Population

The observational AGHLS started in 1977 and initially consisted of 450 boys and girls at the age of 13[31]. Over the last 30 years, 10 follow-up rounds have taken place, resulting in a large database on, for example, anthropometric (body height, body weight, and skinfolds), biological (serum sample variables, blood pressure, and physical fitness), lifestyle (nutritional habits, smoking behavior, and daily physical activity), and psychological variables (coping style, mental wellbeing, vital exhaustion). In the most recent round of measurement (2006) at the age of 42 years, in addition to the regular measurements, microvascular function was assessed in 259 subjects (116 men, 143 (premenopausal) 4 women). The study was approved by the Medical Ethics Committee of the VU University Medical Center and all subjects gave their written informed consent.

Microvascular Function

Nailfold capillary videomicroscopy (Capiscope ®, KK technologies, Devon, UK) was used to obtain information on microvascular function. Capillaries were visualized in the nailfold of the dorsal skin of the third finger with a system magnification of 100·, linked to a personal computer. With this technique, all capillaries that are erythrocyte perfused are visualized. For all subjects, two separate fields of 1 mm^2 were recorded on videotapes. Per field, three measures were taken. At first, baseline capillary density was visualized counting capillaries that were constantly perfused for 15 seconds in resting state. By counting the maximum number of perfused capillaries after four minutes of arterial occlusion (300 mmHg), peak reactive hyperemia was measured, which is considered to be a test of microvascular function. A microvascular recruitment value was calculated by subtracting baseline capillary count from the absolute number of perfused capillaries after arterial occlusion. Finally, after one minute of venous occlusion (50 mmHg), the maximal number of erythrocyte perfused capillaries was measured. Venous occlusion is supposed to reflect structural capillary density[29]. All measurements were separated by a five-minute rest period, and capillary density was reported as the number of perfused capillaries/mm^2. In the literature, other microvascular variables such as percentage recruitment ((peak reactive hyperemia) baseline) / baseline) x 100) or venous percentage recruitment ((venous) baseline) / baseline) x 100) have been used[29,30]. For comparison, these measures were also calculated using obtained measures.

Restricting conditions to measure microvascular variables in participants were a minimum hand temperature of 20°C, fasting state, and at least 30 minutes rest before the measurement as this could possibly influence microvascular perfusion. If hand temperature dropped below 28°C before or during the measurement, subjects were excluded from further analyses.

Reproducibility of the microvascular variables was tested using ICCs. In our hands, intra-observer ICCs of capillary density counts during baseline and after four minutes of arterial occlusion were 0.97 and 0.96 and inter-observer ICCs were 0.86 and 0.90, respectively.

Body Fatness and Body Fat Distribution

In the AGAHLS, body fatness and body fat distribution are measured in different ways. BMI was calculated with weight and height using standard equipment. Waist circumference was measured with a flexible steel tape at the level midway between the lowest rib margin and the iliac crest. A whole body dual-energy X-ray absorptiometry (DEXA) scan was made in 2000 as well as 2006 to quantify total and trunk fatness in kilograms (Hologic 4500, software version 8.21, Hologic, Brussels, Belgium). Total fatness, trunk fatness, and the ratio of trunk/total fatness were used.

Cardiovascular Risk Factors

Blood pressure was measured in a supine position using an automated device (Dinamap Procare 100; GE Healthcare, Boehringher-Ingelheim Germany), at five-minute intervals, for 60 minutes. Fasting high-density lipoprotein (and total) cholesterol, triglycerides, and HbA1c% levels were measured by enzymatic or hexokinase techniques (Roche Diagnostics, Mannheim, Germany).

Data on family history of diabetes and CVD, medication use, physical activity, and smoking were obtained with validated questionnaires[31]. Participants using drugs (potentially) acting on the cardiovascular system were excluded (N = 7: drugs for rate/rhythm control, cholesterol- and blood pressure lowering).

Statistical Analyses

All variables were checked for skewness. Group differences were tested using routine parametric and nonparametric tests, as appropriate. To examine the relationship between microvascular function, and body fatness and body fat distribution, multiple linear regression analyses were used. We analysed the fatness parameters of 2006 (purely cross sectional), the mean values of 2000 and 2006 (cumulative fatness estimate), and the change in fatness parameters from 2000 to 2006. First, crude (univariate) models for the relationship between microvascular function, and body fatness and body fat distribution were computed. Secondly, an adjusted model for gender solely

was constructed. A third model adjusted for gender and CVD risk factors. The fourth and final model included covariates such as family history of diabetes or CVD, physical activity, and smoking. Relationships were tested for effect modification by gender.

To examine a possible nonlinear relationship of body fatness and body fat distribution with microvascular recruitment, linear regression analyses were also performed with gender-specific quartiles of body fatness and body fat distribution as predictor-variables.

All statistical analyses were performed with PASW Statistics 18.0 (PASW statistics Inc., Chicago, Il). A cut-off point for significance of $p < 0.05$ was used.

Results

Characteristics of the AGAHLS population in 2006 are given in Table 3. On average, the population is of normal weight, normotensive, and showed normal levels of blood lipids and HbA1c. Gender differences were present in several fatness parameters. CVD risk factors were not markedly different between male and female subjects.

In Table 4, descriptive information on microvascular function is presented. There is a significant difference between men and women in all measures of microvascular function except baseline perfusion.

Table 5 shows the results of the regression analyses for the 2006 fatness estimates. The univariate relationships of microvascular function with total fat percentage ($b = 0.43$; $p = 0.002$) and trunk over total fatness ratio ($b = 26.23$; $p = 0.03$) were significant. Adjustment for gender, however, explained a large part of these relationships. Effect modification by gender was not apparent (data not shown). Additional adjustment for CVD risk factors, known family history of diabetes, physical activity, or smoking did not change the results (data not shown). Analyses using other measures of microvascular function (i.e., baseline capillary density, percentage recruitment, and venous percentage recruitment) show similar results. Replacing fatness estimates obtained in 2006 by the mean fatness estimates from 2000 and 2006 yielded essentially identical results, as did analysis of fatness measures in quartiles to detect possible nonlinear associations, which are presented in Table 6. Table 6 summarizes univariate relationships of gender-specific quartiles of body fatness (as independent) with microvascular function (as dependent). In this analysis, no threshold effects could be detected.

Table 7 shows the relationships of changes in fatness estimates between 2000 and 2006 with microvascular function. However, in the cross-sectional analyses, total fatness and trunk fatness were significantly associated with microvascular function; changes in total fatness ($b = 1.52$; $p = 0.21$) and changes in trunk fatness ($b = 0.11$; $p = 0.79$) were not.

Table 3. Descriptive information of the 2006 AGAHLS follow-up.

Descriptive statistics	**Male** (N =116)	**Female** (N =143)
	Mean±SD	**Mean±SD**
A. Body fatness and body fat distribution		
BMI (kg/cm2)	25.2±2.9	23.8±3.3
Waist (cm)	83.5±10.8	84.0±10.4
Total body fatness by DXA (kg)	20.3±6.0	22.4±6.8
Trunk fatness (kg)	10.1±3.8	9.2±3.7
Fatness percentage (%)	23.4%±4.6	31.7%±5.5
Trunk- over total fat ratio by DXA	0.49±.06	0.40±.06
B. CVD risk factors		
Systolic blood pressure (mmHg)	122.5±12.8	110.4±12.9
HDL Cholesterol (mmol/L)	1.7 (1.4- 2.0)	1.6 (1.4–1.9)
Triglycerides (mmol/L)	0.9 (0.7-1.3)	0.9 (0.7- 1.3)
HbA1c (%)	5.4±0.3	5.4±0.3
Physical activity (% active below mean)	51.3 %	55.9 %
Smoking (%)	16.4 %	13.3 %
Family history of CVD (%)	56.9 %	69.9 %
Family history of Diabetes (%)	12.1 %	16.8 %
Medication use (%)	6.5 %	4.0 %
Metabolic syndrome >3 characteristics by NCEP	0%	3,4%

Continuous data is presented as mean ± SD or, if skewed, as median (IQR); Dichotomous data is presented as percentage.

Table 4. Descriptive information of microvascular function.

Descriptive statistics	**Male** (N =116)	**Female** (N =143)
	Mean±SD	**Mean±SD**
Baseline capillary density	40.8±15.9	41.2±14.6
Peak Reactive Hyperaemia (PRH)	74.9±21.5	82.2±22.1*
Venous occlusion	79.7±21.9	87.4±21.7*
Recruitment (PRH-baseline)	34.1±12.8	40.9±15.0*
Percentage PRH recruitment (PRH-baseline)/baseline	87.5 (59.6 – 118.9)	96.2 (71.0 – 140.9)*
Percentage Venous recruitment (venous-baseline)/baseline	101.1 (67.7 – 126.5)	108.7 (81.2 – 156.7)*

Data is presented as mean ± SD or, if skewed, as median (IQR); *Indicate a significant difference between males and females, using a t-test.

Table 5. Descriptive information of microvascular function.

	Baseline capillary density			Recruitment		
A. Model 1. Univariate analyses	**β**	**95% CI**	**p**	**B**	**95% CI**	**p**
BMI	-0.28	-0.86 – 0.30	0.35	0.11	-0.45 –0.66	0.71
Waist circumference	0.00	-0.02 – 0.02	0.98	0.01	-0.01 –0.03	0.27
Total fat (kg)	-0.02	-0.31 – 0.27	0.90	0.25	-0.03 – 0.52	0.08
Trunk fat (kg)	-0.18	-0.68 – 0.32	0.48	0.13	-0.35 – 0.60	0.60
Total fat percentage	-0.05	-0.33 – 0.24	0.75	0.43	0.16 – 0.69	0.002*
Trunk/total fatness ratio	-20.6	-45.9 – 4.7	0.11	-26.2	-50.2 – -2.2	0.032*
Cumulative total fatness ((2000 + 2006)/2)	0.05	-0.22 – 0.32	0.72	0.36	0.10 – 0.61	0.007*
Cumulative trunk fatness ((2000 + 2006)/2)	0.05	-0.29 – 0.40	0.76	0.30	-0.04 – 0.64	0.08
B. Model 2. Adjusted for gender	**β**	**95% CI**	**P**	**B**	**95% CI**	**p**
BMI	-0.28	-0.89 – 0.32	0.32	0.11	-0.44 – 0.66	0.69
Waist circumference	0.00	-0.02 – 0.02	0.81	0.01	-0.01 – 0.03	0.34
Total fat (kg)	-0.02	-0.31 – 0.27	0.87	0.26	-0.14 – 0.53	0.07
Trunk fat (kg)	-0.18	-0.68 – 0.33	0.50	0.18	-0.29 – 0.66	0.45
Total fat percentage	-0.05	-0.33 – 0.24	0.75	0.15	-0.19 – 0.49	0.30
Trunk/total fatness ratio	-27.1	-57.5 – 3.4	0.08	-0.06	-28.4 – 28.2	0.99
Cumulative total fatness ((2000 + 2006)/2)	0.05	-0.22 – 0.32	0.72	0.03	-0.15 – 0.21	0.74
Cumulative trunk fatness ((2000 + 2006)/2)	0.05	-0.29 – 0.40	0.76	0.03	-0.17 – 0.23	0.77

β = regression coefficient, 95%CI = 95% confidence interval and p indicates the significance-level of the relationship. * Indicate a significant association ($p \leq 0.05$).

Table 6. Regression analyses, with gender-specific quartiles of body fatness and body fat distribution.

	Recruitment		
Quartiles of:	**β**	**95% CI**	**p**
BMI			
Reference group Q1			
- Q2 versus Q1	-0.68	-5.67 – 4.32	0.79
- Q3 versus Q1	3.28	-1.72 – 8.27.	0.20
- Q4 versus Q1	-0.12	-5.13 – 4.90	0.96
Total fat percentage			
Reference group Q1			
- Q2 versus Q1	2.13	-2.82– 7.08	0.40
- Q3 versus Q1	-2.48	-7.43 – 2.47	0.33
- Q4 versus Q1	3.14	-1.81 – 8.09	0.21
Trunk/total fatness ratio			
Reference group Q1			
- Q2 versus Q1	2.13	-2.82 – 7.08	0.40
- Q3 versus Q1	-2.48	-7.43 – 2.47	0.33
- Q4 versus Q1	3.14	-1.81 – 8.09	0.21

β = difference in microvascular function for the quartile compared to reference quartile (Q1), 95%CI = 95% confidence interval and p indicates the significance-level of the relationship.

This summarizes univariate relationships of gender specific quartiles of body fatness (as independent) with microvascular function (as dependent).

Table 7. Regression analyses with change in fatness between 2000 and 2006.

	Baseline capillary density			Recruitment		
A. Model 1. Univariate analyses	**β**	**95% CI**	**p**	**B**	**95% CI**	**p**
BMI (Δ 2000 – 2006 as %)	0.00	-0.31 – 0.31	0.99	0.25	-0.05 – 0.55	0.10
Waist circumference (Δ 2000 – 2006 as %)	0.05	-0.08 – 0.19	0.45	0.22	0.09 – 0.35	0.01*
Change in total fatness (Δ 2000 – 2006 as %)	-1.52	-3.87 – 0.83	0.21	-1.19	-3.49 – 1.12	0.31
Change in trunk fatness (Δ 2000 – 2006 as %)	0.11	-0.67 – 0.88	0.79	-0.43	-1.23 – 0.37	0.29
Total fat percentage (Δ 2000 – 2006 as %)	-0.28	-0.11 – 0.05	0.50	0.01	-0.66 – 0.09	0.75
Trunk/total fatness ratio(Δ 2000 – 2006 as %)	0.29	-0.31 – 0.37	0.86	0.02	-0.35 – 0.39	0.90
B. Model 2. Adjusted for gender	**β**	**95% CI**	**P**	**B**	**95% CI**	**p**
BMI (Δ 2000 – 2006 as %)	-0.00	-3.86 – 3.77	0.99	0.27	-0.03 – 0.56	0.07
Waist circumference (Δ 2000 – 2006 as %)	0.05	-0.83 – 0.19	0.45	0.21	0.09 – 0.34	0.01*
Change in total fatness (Δ 2000 – 2006 as %)	-1.55	-3.91 – 0.82	0.20	-0.98	-3.27 – 1.13	0.40
Change in trunk fatness (Δ 2000 – 2006 as %)	0.04	-0.73 – 0.82	0.91	0.23	-0.61 – 1.08	0.58
Total fat percentage (Δ 2000 – 2006 as %)	-0.28	-0.11 – 0.05	0.49	-0.33	-1.11 – 0.44	0.66
Trunk/total fatness ratio (Δ 2000 – 2006 as %)	0.29	-0.31 – 0.37	0.86	0.04	-0.33 – 0.41	0.82

β = regression coefficient, 95%CI = 95% confidence interval and p indicates the significance-level of the relationship. * Indicate a significant association ($p \leq 0.05$).

Discussion

The current study in predominantly normal weight individuals does not support the existence of linear relationship between baseline capillary density and capillary recruitment on the one hand, and measures of body fatness and body fat distribution, on the other. Also, changes in fatness over a six-year period were not associated with measures of microvascular function. A novel finding was a significant difference in microvascular function between men and women.

It is important to note that the range of body fatness, as well as the degree by which fatness parameters changed over the six-year period, is narrow in this relatively healthy population. The mean BMI and other CVD risk factors, as shown in Table 3, are comparable to the healthy Dutch population[191,192]. An association between fatness and microvascular function may become apparent only in more extreme phenotypes of (central) obesity and / or in those who show marked changes in fatness. A clear difference in microvascular function was, for example, found in a case–control study comparing subjects with a mean BMI of 38.5 versus 21.3 kg/m2 in controls[31], which may suggest a threshold effect. Our current results at least suggest that any association between fatness and microvascular function is unlikely to be linear and to 8 extent into the relatively normal range. No threshold effect could be detected in this population (as illustrated in Table 6). Of course, this does not exclude a possible threshold effect for the body fatness values, which lie outside the range of our population.

A novel finding was a consistent difference between men and women in functional and structural measures of microvascular function (i.e., women score approximately 7 capillaries /mm2 higher, except baseline capillary density). The levels of baseline capillary density in this study are consistent with previous smaller studies, which do not show baseline difference either, even when using more extreme phenotype populations[6]. These gender differences not only confounded the univariate associations between fatness and the microcirculation in our study, but they are also important to take into account in future studies addressing skin capillary density. For measures of body fatness and body fat distribution, it is widely known that men and women differ. In the present study, women have a slightly lower BMI, which seems to be in contrast with the total Dutch population[193]. However, we believe that this non-significant difference is probably a chance finding, relating to the normal and relatively narrow range of values of BMI. All women in this population are premenopausal. Furthermore, we previously found that menstrual cycle does not influence microvascular function[192]. Also, 10 no difference in HDLc was observed between sexes, whereas this could have been expected. Again, however, the ranges are narrow, the confidence intervals overlap markedly, and the study was not designed to address gender specific lipid profiles.

In our analyses, a wide range of indices of body fatness and body fat distribution was used, but the relative importance of these different measures is not always clear[20–22], In the final adjusted model, none of the indices of body fatness or body fat distribution was associated with microvascular function. Further research is needed to examine different obesity-induced effects on microvascular function obtained with measures of body fatness and body fat distribution.

In the current study, the non-invasive method of skin capillaroscopy was used to asses microvascular function. Measuring microvascular function in skin as a proxy for microvascular function in muscle is a limitation of this technique. This limitation is acknowledged, but there is a wealth of publications suggesting that microvascular function assessed in skin is representative of microvascular function in other organs, for example, muscle perfusion and exchange capacity[4,6,29,30]. Moreover, in contrast to most methods used to assess microvascular function, this technique is suitable to be used in large scale studies.

The literature contains two comparable studies providing additional information on obesity-related microvascular dysfunction. First, a recent study by Czernichow et al. Studied similar relations in a comparably large study population of healthy insulin-sensitive, but overweight men and women, and found body fatness to be inversely associated with microvascular function[29]. This population, in comparison with that of the current study, consisted of older and more extreme phenotype subjects. In addition, overweight subjects in this study were selected based on normal insulin sensitivity, which may not be representative of a non-selected overweight population. Methods used in this study for body fatness were bio-impedance and waist circumference, both fairly robust measures for the estimation of body fatness, but different from our more direct assessments by DEXA. Methods for measuring microvascular function were in part comparable, but only included perfused capillary count during venous congestion, not the more widely used peak after arterial occlusion. The results in the current study were essentially identical when peak reactive hyperemia was replaced by venous recruitment as used by Czernichow et al.[29] (data not shown). Hence, the Chernikow study suggests that a negative effect of body fatness and body fat distribution on microvascular function may become apparent in an older and more overweight population. The second comparable study was conducted by Irving et al.[30] In that study, a relationship of microvascular function with BMI was shown. However, that study was restricted to male subjects, selected from a four-corner design based on parental and own blood pressure[30]. This could possibly affect the healthiness of the population and/or increase the number of extreme phenotype individuals. Furthermore, other variables of body fatness and body fat distribution were not obtained.

In conclusion, there appears to be no linear relationship between body fatness or body fat distribution, and microvascular function in a healthy

middle-aged population. This does not suggest that previously reported associations between fatness and microvascular function are false, but rather that the association may depend on patient selection or on a particular threshold of fatness, above which microvascular function becomes perturbed. In addition, our study shows a difference in microvascular function for men and women. Future studies should take the demonstrated gender differences into account.

Perspective

Unravelling the aetiology of obesity induced microvascular (dys-)function has, so far, lead to the idea of a linear relationship of body fatness and body fat distribution with microvascular function. Hence, the higher fatness, the lower microvascular function. This study examines the relationship in healthy subjects. Unfortunately, this epidemiological study cannot confirm obesity induced effects on microvascular function within the healthy range.

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