The relationship between body fatness, blood pressure and insulin resistance: a mediating role for microvascular function?

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Introduction

Obesity has been implicated in the rising prevalence of the metabolic syndrome, a cluster of risk factors including, hypertension, manifestations of insulin resistance, and dyslipidemia. These clustered risk factors confer an increased risk for type 2 diabetes and cardiovascular disease (CVD)[6,8,78]. Although associations of obesity with hypertension and insulin resistance are well described, the underlying mechanisms are poorly understood. Obesity-associated microvascular dysfunction is hypothesized to explain part of these associations[8]. Microvascular dysfunction, by affecting both flow resistance and nutrient delivery, seems important in the development of hypertension and insulin resistance, a prelude to type 2 diabetes. Indeed, microvascular dysfunction can be demonstrated in obese subjects[6], subjects with untreated hypertension[10], and subjects with impaired glucose metabolism[168]. Whereas these small studies of highly selected subjects suggest that dysfunction of the microvasculature at the level of both resistance vessels and the nutritive capillary beds develops progressively along with an increase of adiposity, previous population based studies show controversies with regard to

the linear assumption in this hypothesis[29,30,55]. Up till now, three studies investigated larger populations. Two studies confirmed a linear relationship of body fat with microvascular function, but selected subjects on the basis of being overweight or from a four corner model based on the subject's own and parental blood pressure[29,30]. A recent study by our group in a young and very healthy population not selected on the basis of fatness parameters, blood pressure or insulin sensitivity, could not confirm this linear relationship[55]. The current observational study, performed in an older and more metabolically perturbed population-based cohort, in which subjects were selected on the basis of their glucose tolerance status, examined the potential existence of relationships among body fatness, body fat distribution, blood pressure and insulin resistance with microvascular function in individuals with normal and impaired glucose metabolism. Furthermore, the mediating role of microvascular function in the relationship of body fat with both blood pressure and insulin sensitivity is investigated.

Methods

Study population

In 2010, a random sample of 250 subjects was selected from the New Hoorn Study population, described in detail elsewhere[27]. In the first round of measurement in 2006, 2753 subjects completed an oral glucose tolerance test (OGTT)[23]. The results of the OGTT in 2006 were used for the selection procedure in 2010. An invitation was send to one hundred twenty-five subjects with normal glucose tolerance (NGT) in 2006 and one hundred twenty-five subjects with impaired glucose metabolism (IGM: both IFG and IGT) in 2006. Subjects with type 2 diabetes mellitus (T2DM) in 2006 were excluded. Eventually, 105 NGT and 100 IGM subjects responded and were examined in 2010. These selected subjects underwent a repeated OGTT in 2010, and were reclassified in NGT, IGM and T2DM according to this test.

All subjects provided a written informed consent and the study was approved by the medical ethics committee of the VU University Medical centre Amsterdam.

Microvascular function

Microvascular function was assessed by capillary videomicroscopy (Capiscope®, KK technologies, Devon, UK) at the dorsal skin of the nailfold of the middle finger. Methods are described in detail elsewhere[55]. Briefly, nailfold capillaries in the dorsal skin of the middle finger were visualized by a capillary microscope with a system magnification of 100x. Capillary density was defined as the number of erythrocyte-perfused capillaries per mm^2. Baseline capillary density (BCD) was measured by counting all capillaries that are constantly perfused during 15seconds in rest. Capillary density during peak reactive hyperemia (PRH) was defined as maximal perfused capillaries after

4 minutes of arterial occlusion. All measures were separated with a 5 minute rest-period and were performed in duplicate. Restricting conditions were a minimum skin temperature of 28°C, fasting state and at least 30 minutes rest before the measurement. Calculated scores were recruitment, (peak reactive hyperemia minus baseline perfusion) and percentage recruitment (peak reactive hyperemia / baseline).

Reproducibility of the microvascular variables were tested using intra-class correlation coefficients (ICCs). With 4 observers, inter-observer ICCs of capillary density counts during baseline and after 4 minutes of arterial occlusion were 0.84 and 0.94 respectively.

Body fatness parameters

Body weight and height were measured using standardized techniques[23]. Height and weight were measured without shoes and heavy clothes, using a Seca 888 compact digital flat scale (Vogel & Halke, Hamburg, Germany) and a stadiometer (holtain, London, UK). BMI was calculated as weight in kilograms divided by the square of height in meters.

Additional information on anthropometrics was obtained using a Lunar prodigy pro dual energy X-ray absorptionmetry (DEXA) scan (GE Lunar, Madison, WI,USA). A DEXA scan allows for a precise estimation of lean, fat and bone mass and subsequent proportion of the total mass.

Biological CVD risk factors

Systolic and diastolic blood pressure (SBP and DBP) were measured three times in the supine position, on a 10 minute interval on the left arm using a Colin Press Mate BP 8800 (Colin electronics, Hayashi Komaki, Japan).

All serum analyses were performed at the clinical chemistry laboratory of the VU Medical Center Amsterdam. Glucose was measured in venous plasma by the glucose oxidase method (gluco-quant/hexokinase/G6P-DH (Boeringher Mannheim, Mannheim Germany). Hba1C was assessed using Diabetes Control and Complications Trail (DCCT) standardized reverse-phase cation exchange chromatography (HA 8160 analyzer; Menarini, Florence, Italy). Total and high density lipoprotein cholesterol, triglycerides, ALAT and ASAT levels were measured by enzymatic or hexokinatic techniques (Roche Diagnostics, Mannheim, Germany). Insulin levels were measured using an ILMA assay (Centaur). HOMA-IR was calculated as (fasting insulin X fasting glucose) / 22.5. Due to skewness the natural logarithm (ln) of HOMA-IR was used in further analyses.

Categorical glucose metabolism status was defined according to the World Health Organization 2006 criteria[239] and includes subjects with normal glucose metabolism (NGT), with impaired glucose metabolism (IGM on either fasting (IFG) or 2 hours glucose levels(IGT)) and with type 2 diabetes (DM). Cut-off points that were used for fasting plasma glucose are NGT <6.1

mmol/L; IFG 6.1-7.0 mmol/L; DM>7.0 mmol/L and for 2 hours plasma glucose: NGT<7.8; IGT 7.8-11.1 mmol/L; DM >11.1 mmol/L.

Statistical analyses

First, all variables were checked for skewness and, if necessary, reported as median and interquartile range (for skewed variables) or percentages (for categorical variables).

To examine the relationships of body fat (independent) with insulin sensitivity, blood pressure, and microvascular parameters, as well as to examine the mediating role of microvascular parameters, linear regression analyses were used. First, crude (univariate) models of body fat with either blood pressure, insulin sensitivity or microvascular recruitment were computed. For the analyses of HOMA-IR, newly discovered diabetes patients based on the OGTT in 2010 were excluded[30]. To exclude complex confounding, even in the absence of statistically significant univariate associations, the mediating role for microvascular function was examined by using the method of Baron and Kenny, leading to the percentage mediation[240,241]. This was done using the STATA 'medeff'-command and an additional Sobel-test for significance[242]. This analysis results in a percentage (E) and is an estimation of how much of the relationship between fatness parameters and certain cardiovascular risk factors can be explained by microvascular recruitment.

Confounding was checked using age, medication use, cholesterol levels, blood pressure or insulin sensitivity (depending on the dependent variable) and triglyceride levels, as possible confounders in the final fully adjusted model.

All statistical analyses were performed separately for males and females. Analyses were performed with PASW Statistics 21.0, PASW Statistics Inc., Chicago, IL, USA. The mediation analyses were performed using STATA (STATA version 11. StataCorp LP, Texas, USA). A cut-off point for significance of $p < 0.05$ was used.

Results

Descriptive information of the population is shown in Table 16 for males and females separately. Table 17 shows descriptive information on microvascular variables for the separate clinical glucose tolerance groups (e.g. NGT, IGM and DM). Male subjects with impaired glucose metabolism show lower percentage capillary recruitment compared to NGT (34.0% versus 47.7%) which seemed to be explained mainly by a higher BCD (45.3 n/mm^2 versus 42.9 n/mm^2) rather than an evident difference in PRH (62.9 n/mm^2 versus 62.8 n/mm^2). In total, 62 subjects were on antihypertensive therapy; 20% of NGT-subjects, 31% of IGM, and 48% of T2DM subjects.

Univariate relationships among body fatness parameters, blood pressure, insulin resistance, and microvascular variables are presented in Table 18.

For models including ln HOMA-IR 15 newly discovered DM men and 10 newly discovered DM women were excluded. Table 19 shows the same data as in table 18A, but corrected for possible confounding factors such as age, medication use, cholesterol levels, triglyceride levels, blood pressure, and insulin resistance. Only the associations between body fatness and insulin resistance remained statistically significant.

The result of the mediation analyses concerning insulin resistance (ln HOMA-IR) and systolic blood pressure (SBP) are presented in Table 20. No mediating role of microvascular function could be detected in the relationship between measures of body fatness on the one hand, and insulin resistance and blood pressure on the other.

Table 16. Descriptive information of the current sample from the New Hoorn population.

	Males N=120	**Females N=85**
Biological CVD risk factors		
Age (years)	58.6 ± 6.47	56.4 ± 5.93
Glucose tolerance		
%IGM	38.1%	26.8%
%DM	12.7%	12.2%
Glucose 0hrs (mmol/L)	6.1 ± 0.63	5.7 ± 0.57
Glucose 2hrs (mmol/L)	6.2 (5.3 to 7.4)	6.5 (5.4 to 8.2)
HOMA-IR	2.9 (1.9 – 4.4)	2.1 (1.3 to 3.3)
Systolic Blood Pressure (mmHg)	139 ± 17.9	129 ± 15.9
Diastolic Blood Pressure (mmHg)	81 ± 10.9	72 ± 9.2
Triglycerides (mmol/L)	1.5 (0.4-6.1)	1.2 (0.3-7.2)
Total Cholesterol (mmol/L)	5.6 ± 1.07	6.0 ± 1.19
HDL Cholesterol (mmol/L)	1.3 ± 0.38	1.7 ± 0.41
Anti-hypertensive usage (N)	34	28
Descriptive information is presented as Mean ± Standard Deviation or median (IQR) when appropriate.		
Microvascular function		
BCD (n/mm2)	44 ± 10.2	42 ± 10.3
PRH (n/mm2)	63 ± 15.6	66 ± 14.5
Recruitment (n/mm2)	19 ± 9.7	25 ± 11.4
Percentage recruitment (%)	41.7 (3.6-107.1)	59.3 (2.5-148.6)
Body fatness		
Total fat (kg)	29 ± 6.1	39 ± 6.8
Trunk fat (kg)	17 ± 5.4	16 ± 5.6
Trunk over total fatness (kg/kg)	0.56 ± 0.091	0.39 ± 0.090
BMI (kg/m2)	28.0 ± 3.56	26.6 ± 4.67
FAT percentage (%)	30.3 ± 6.24	40.2 ± 7.17

Table 17. Descriptive information on microvascular function according to glucose status

Glucose tolerance status **Microvascular variable**	**NGT** M n=58 / F n=50	**IGM** M n=45 / F n=22	**DM** M n=15 / F n=10
BCD	42.9(9.4) /40.6(8.4)	45.3 (11.3) / 43.1 (12.9)	42.4(9.3) / 44.4(14.4)
PRH	62.9(16.8) / 65.8(13.9)	62.8 (15.2) / 67.5 (16.0)	62.0(10.7) / 68.0(18.0)
Recruitment (n/mm^2)	20.3(9.7) / 25.2(11.3)	17.2(9.1) / 24.4(12.8)	19.6(11.3) / 23.4(9.1)
Percentage recruitment (%)	47.7 / 61.0 (4.6-107.1) / (2.5-139.5)	34.0 / 52.1 (5.8-74.7) / (2.5-148.6)	47.2 / 65.6 (3.6-103.5) / (8.3-102.0)

Data is presented as mean (SD) or median (IQR).
Numbers in the table are based on subjects with both a completed microvascular and glucose tolerance measurement.

Table 18. Results from univariate regression analyses

A. Associations of body fatness with blood pressure and insulin resistance

	Body fat percentage		**BMI**	
Gender Dependent variable	Male	Female	Male	Female
Systolic blood pressure	0.57 * (0.05 – 1.07)	0.29 (-0.19 – 0.78)	0.98 * (0.08 – 1.88)	0.57 (-0.17 – 1.310)
Diastolic blood pressure	0.37* (0.06 – 0.68)	0.26 (-0.02 – 0.54)	0.66* (0.11 – 1.20)	0.45* (0.03 – 0.88)
Ln HOMA-IR	0.16 * (0.10 – 0.21)	0.13 * (0.05 – 0.21)	0.28 * (0.18 – 0.38)	0.31* (0.20 – 0.43)

B. Associations of body fatness with microvascular function

	Body fat percentage		**BMI**	
Gender Dependent variable	Male	Female	Male	Female
BCD	0.14 (-0.17 – 0.46)	-0.09 (-0.41 – 0.23)	0.21 (-0.06 – 1.06)	-0.15 (-0.66 – 0.37)
PRH	-0.07 (-0.42 – 0.56)	0.17 (-0.29 – 0.62)	0.12 (-0.76 – 0.99)	0.21 (-0.53 – 0.93)
Recruitment	-0.07 ((-0.38 – 0.23)	0.26 (-0.09 – 0.62)	-0.41 (-0.95 – 0.13)	0.36 (-0.21 – 0.93)
Percentage recruitment	-0.19 (-0.90 – 0.51)	0.85 (-0.14 – 1.84)	-1.32 * (-2.56 - -0.08)	1.33 (-0.27 – 2.92)

C. Associations of blood pressure and insulin resistance with microvascular function

	Blood pressure **Systolic** **Diastolic**		**Ln HOMA-IR**	
Gender Dependent variable	Male	Female	Male	Female
BCD	0.04 (-0.08 – 0.14) *0.09* *(-0.10 – 0.27)*	0.12 (-0.03 – 0.27) *0.17* *(-0.07 – 0.41)*	1.01 (1.00 – 1.02)	1.00 (0.99 – 1.02)
PRH	-0.04 (-0.21 – 0.13) *0.02* *(-0.27 – 0.30)*	0.14 (-0.07 – 0.36) *0.12* *(-0.22 – 0.47)*	1.01 (1.00 – 1.01)	1.01 (1.00 – 1.02)
Recruitment	-0.05 (-0.16 – 0.05) *-0.10* *(-0.28 – 0.08)*	0.02 (-0.15 – 0.19) *-0.05* *(-0.32 – 0.23)*	0.99 (0.98 – 1.01)	1.01 (1.00 – 1.02)
Percentage recruitment	-0.14 (-0.39 – 0.10) *-0.31* *(-0.73 – 0.10)*	0.05 (-0.43 – 0.53) *-0.08* *(-0.86 – 0.70)*	0.99 (0.98 – 1.00)	1.01 (1.00 – 1.01)

Values are β (CI) for analyses with blood pressure, for analyses with lnHOMA-IR, exp(B) and CI are presented. BCD = baseline capillary density; PRH = peak reactive hyperemia. Significant associations are marked with an * for $p<0.05$.

Table 19. Adjusted associations of body fatness with blood pressure and insulin resistance

	Body fat percentage		**BMI**	
Gender Dependent variable	Male	Female	Male	Female
Systolic blood pressure	0.52 (-0.17 – 1.20)	0.03 (-0.49 – 0.54)	0.59 (-0.65 – 1.83)	-0.38 (-1.33 – 0.57)
Diastolic blood pressure	0.31 (-0.11 – 0.72)	0.11 (-0.20 – 0.43)	0.56 (-0.19 – 1.31)	0.04 (-0.52 – 0.60)
Ln HOMA-IR	0.15 * (0.05 – 0.15)	0.08 * (0.08 – 0.17)	0.22* (0.13 – 0.320)	0.17* (0.11 – 0.24)

Values are β (CI) and significant associations are marked with an * for $p<0.05$.
Confounders are: age, medication use, cholesterol levels, triglyceride levels, and blood pressure or insulin sensitivity (depending on the dependent variable).

Table 20. Results from mediation analyses

A. Mediation analyses estimating the mediating effect of BCD

	Body fat percentage		**BMI**	
Gender Dependent variable	M	F	M	F
Ln HOMA-IR	N= 96 E = 1.3% P = 0.53	N= 75 E = -1.9% P = 0.59	N= 96 E = 2.0% P = 0.51	N= 75 E = -1.6% P = 0.57
SBP	N = 98 E = 2.2% P = 0.45	N = 77 E = -8.0% P = 0.62	N = 99 E = 4.0% P = 0.74	N = 76 E = -7.5% P = 0.59

B. Mediation analyses estimating the mediating effect of PRH

	Body fat percentage		**BMI**	
Gender Dependent variable	M	F	M	F
Ln HOMA-IR	N= 95 E = 0.3% P = 0.87	N= 74 E = 3.5% P = 0.55	N= 95 E = 0.2% P = 0.92	N= 74 E = 1.8% P = 0.65
SBP	N = 98 E = -0.6% P = 0.80	N = 75 E = 7.2% P = 0.53	N = 98 E = -0.5% P = 0.81	N = 75 E = 5.3% P = 0.61

C. Mediation analyses estimating the mediating effect of percentage recruitment

	Body fat percentage		**BMI**	
Gender Dependent variable	M	F	M	F
Ln HOMA-IR	N= 93 E = -0.4% P = 0.63	N= 74 E = 6.4% P = 0.31	N= 93 E = -3.0% P = 0.60	N= 74 E = 3.6% P = 0.35
SBP	N = 96 E = 2.1% P = 0.63	N = 75 E = 1.1% P = 0.96	N = 96 E = 6.2% P = 0.44	N = 75 E = 1.1% P = 0.95

BCD = baseline capillary density; PRH = peak reactive hyperemia; N = number of observations; E = Percentage total effect mediated; P = significance level of the Sobel test.

Discussion

Obesity-related abnormalities of microvascular structure and function have previously been associated with conditions such as high blood pressure, insulin resistance and type 2 diabetes[8]. In the present study, body fatness, indexed by body fat percentage and BMI, was positively related to blood pressure and insulin resistance, as was expected, although after adjustment for potential confounders only the association between body fatness and insulin resistance remained. Microvascular function, expressed as percentage capillary recruitment after arterial occlusion, was inversely associated with BMI only in males. None of the microvascular function variables were significantly associated with blood pressure or insulin resistance in both sexes. No mediating role of percentage capillary recruitment in the relationship between body fatness, and both insulin resistance and blood pressure could be demonstrated. In addition, the present study could confirm the earlier observed sex difference in microvascular structure and function[55].

Whereas a large body of evidence, mostly obtained in small, experimental studies, has shown that obesity, hypertension and diabetes are characterized by impaired microvascular function[4,6,9,14,97,] the present study in normal and impaired glucose metabolism could demonstrate only a weak inverse relationship between BMI and percentage capillary recruitment in males. In contrast to earlier findings[6,10,168,] this decrease in percentage capillary recruitment was not determined by a decrease in capillary density during peak reactive hyperemia, but merely by a combination of non-significant increase in baseline capillary density and a comparable capillary density during peak reactive hyperemia. The lack of a relationship between measures of insulin resistance and microvascular function in our cohort also contrasts with previous studies using the euglycemic, hyperinsulinemic clamp technique to assess insulin sensitivity[1,4,6,168]. This finding is, however, consistent with most studies using HOMA-IR that could not detect a relationship with capillary density[29,30,55]. Several explanations for these weak relationships may be at play. One explanation might be that the association between body fatness and microvascular function may only become apparent in more extreme phenotypes of (central) obesity, such as examined in most experimental studies[6]. Another explanation could be found in the fact that earlier studies[4,6,10] excluded subjects that used antihypertensive treatment. In our cohort, antihypertensive therapy was used by 20% of NGT, 31% of IGM, and 48% of T2DM subjects, which may have obscured certain associations with microvascular function, because antihypertensive treatment may increase and even normalize capillary density[90,168,243].

Given the weak univariate relationships between microvascular function and measures of body fatness, insulin resistance and blood pressure, the mediation analyses demonstrated, not unsurprisingly, that microvascular

function did neither mediate the relationship between body fatness and insulin resistance nor the relationship between body fatness blood pressure, in both sexes.

Limitations of the study should be mentioned. Although reasonable correlations can be observed between HOMA-IR and clamp-derived insulin sensitivity[13,244,245,] it should be realized that from a pathophysiological standpoint HOMA-IR (hepatic insulin resistance) and clamp-derived insulin sensitivity (peripheral insulin resistance) could provide different information[246]. An additional limitation could be that insulin-mediated effects on capillary density should have been studied when examining the effects of microvascular function on insulin sensitivity[8,168]. Another methodological concern is whether the vascular responses observed in skin reflect those in muscle. However, many data have been reported to suggest that the cutaneous microcirculation is a representative vascular bed to examine interactions between insulin resistance, high blood pressure and generalized microvascular dysfunction[15,247,248]. In addition, we used a surrogate measure of insulin-mediated capillary recruitment, which has been shown to be a determinant of glucose uptake[8]. Although the number of capillaries recruited during PRH, as measured in the present study, was associated with insulin-mediated capillary recruitment, the role of insulin-mediated capillary recruitment remains to be examined more directly in future studies.

In conclusion, body fatness was associated with blood pressure and insulin resistance. Only in males an association was found between body fatness and microvascular function. Microvascular function did neither mediate the relationship between body fatness and insulin resistance nor the relationship between body fatness blood pressure, in both males and females.

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