chapter 7 – vasomotion and adiponectin

Body mass index is related to microvascular vasomotion, this is partly explained by adiponectin

European Journal of Clinical Investigation 44: 660-667, 2014

Michiel P de Boer
Nienke J Wijnstok
Erik H Serné
Etto C Eringa
Coen D A Stehouwer
Allan Flyvbjerg
Trynke Hoekstra
Martijn W Heymans
Rick I Meijer
Jos W Twisk
Yvo M Smulders.

Abstract

Obesity-related microvascular dysfunction, including alterations in rhythmic changes in vascular diameter, so-called 'vasomotion', may be important in the clustering of obesity with other cardiovascular risk factors. Adipokines have been suggested to play a role in obesity-related vascular dysfunction. Alterations in vasomotion have been found using extreme body mass index (BMI) phenotypes. Whether these alterations can be translated to the general population is unknown. The aim was to investigate relationships between BMI, vasomotion and adipokines in a population-based cohort. Body fatness, vasomotion, adiponectin and leptin were determined in 94 healthy participants (age 42 years, 46 men, mean BMI 25.5±3.8 kg·m-2) of the Amsterdam Growth and Health Longitudinal Study (AGHLS). Vasomotion was assessed via wavelet analysis of skin laser Doppler flowmetry (LDF). BMI was associated with the neurogenic domain of the vasomotion spectrum (β -0.011, p=0.046), adiponectin (β -0.18, p=0.028) and leptin (β 2.22, p<0.0001). Adiponectin was positively associated with the neurogenic domain of vasomotion (β 0.016, p=0.019). Leptin did not show any significant relationship with vasomotion. The association between BMI and the neurogenic domain of the vasomotion spectrum was partly explained by adiponectin. The association between body fatness and microvascular vasomotion also applies to the normal population, and is partly explained by adiponectin.

Introduction

Obesity and its sequelae have become a health problem of global proportions (1). The relationship between obesity and cardiovascular risk factors such as hypertension and insulin resistance is well established (2). Although adipokines such as adiponectin are increasingly identified as key players in the association between body fatness and vascular perturbations (3,4,5), the underlying mechanisms are not yet clear. It has been proposed that microvascular dysfunction plays a central role in obesity-related vascular and metabolic sequelae (6,7). Microvascular dysfunction may contribute to both impaired insulin delivery – and hence uptake – in skeletal muscle, as well as to increased vascular resistance and hypertension. An important but understudied component of microvascular function is vasomotion, defined as a rhythmic change of precapillary arteriolar diameter. It ensures spatial and temporal heterogeneity of microvascular perfusion, not only meeting metabolic demands but also affecting peripheral vascular resistance (8). Spectral analysis of these rhythmic changes in arteriolar diameter and the subsequent rhythmic fluctuation in blood flow through blood vessels, as assessed by LDF, identified several different sources of vasomotion, each contributing to a distinct part of the vasomotion frequency spectrum (9,10). To date, our knowledge of the relationship of obesity with vasomotion is mainly based on studies on selected populations with extreme body fatness phenotypes. These studies suggest that the neurogenic and endothelial vasomotion domains are decreased in amplitude in obese subjects (11,12). Whether the differences 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 unknown. Although adipokines are potential modulators of local arteriolar vasomotion, here have been no studies investigating such relationships. In the current observational study, performed in an apparently healthy, population-based cohort, we examined the relationship between adiposity and microvascular vasomotion. Further, we assessed the postulated mediating role of adiponectin in this relationship.

Methods

Reporting of the study conforms to STROBE statement along with references to STROBE statement and the broader EQUATOR guidelines (Simera et al. January 2010 issue of EJCI).

Subjects

The Amsterdam Growth and Health Longitudinal Study (AGHLS) is an observational longitudinal cohort study that began in 1976 with a group of 450 boys and girls. Its initial goals were to investigate longitudinal relationships between biological and lifestyle variables (13). In the most recent measurement round (2006) at the age of 42 years, vasomotion was assessed in skin via LDF. The study was approved by the medical ethical committee of the VU University Medical Center, and all subjects gave their written informed consent. For the current observational study, all the members of the cohort who underwent an LDF measurement (n = 128) were used in the analyses. Subjects with incomplete data on BMI or adipokines (n = 16) and subjects with laser Doppler recordings < 15 min (n = 18) were excluded, leaving 94 subjects for further analyses (table 1). There were no additional exclusion criteria. All measurements were performed on the same day.

Adiposity and body fat distribution

Body fatness and body fat distribution were measured in different ways. Anthropometric measurements of body height, body mass, waist circumference and skinfolds were performed according to standard procedures (14). BMI was calculated by dividing body mass (kg) by body height squared (m^2). Skinfolds (biceps, triceps, subscapular and suprailiac) were measured with a Harpenden caliper (Holtain, Crosswell, UK) to the nearest 0.1 mm according to the recommendations of the International Biological Programme (15). A whole-body dual energy X-ray absorptiometry (DEXA) scan was made to quantify trunk fatness in kilograms (Hologic 4500, software version 8.21, Hologic, Brussels, Belgium).

Adipokines

On the study day, a blood sample was taken from all participants after overnight fasting. Total adiponectin was determined in plasma samples by an in-house time-resolving immunofluorometric assay as described earlier (16). The interassay CV of a human quality control serum sample averaged 9.2%. The intraassay CV of samples analysed in duplicate averaged 2%. Leptin was determined in serum samples via electrochemiluminescence with a 2-plex multi-array (Meso Scale Discovery, MSD, Gaithersburg, MD, USA). The inter- and intra-assay CV were < 5%.

Laser Doppler flowmetry

All measurements were performed in the fasting state in the morning in a quiet temperature-controlled room (23.0 ± 1.0°C). All participants had abstained from caffeine and alcohol-containing drinks or smoking overnight. Measurements were started after a 30-min period for acclimatization and rest. Microvascular measurements were performed in a sitting position with the investigated hand at heart level. Skin temperature was registered continuously and was above 28 °C at the start of all microvascular measurements. Skin blood flow was measured in conventional perfusion units (PU) by means of a laser Doppler system (Periflux 4000; Perimed, Stockholm, Sweden). Microvascular measurements were performed with one thermostatic laser Doppler probe (PF 481; Perimed) positioned at the dorsal side of the distal phalanx of the third digit of the non-dominant hand.

Vasomotion

Wavelet analysis of LDF signals 15 min or more in length (mean 20.3 ± 3.0 min) was conducted to assess the frequency spectrum between 0.01 and 1.6 Hz. The spectrum was divided into the five frequency intervals as described by Stefanovska et al. The first three lower frequency domains are thought to be locally generated (i.e. in arterioles), contributing to local perfusion: 0.01–0.02 Hz, endothelial activity; 0.02–0.06 Hz, neurogenic activity; and 0.06–0.15 Hz, myogenic response of the smooth muscle cells in the vessel wall. The higher frequencies originate upstream, are conducted through the vascular tree and are registered in the periphery: 0.15–0.4 Hz, attributed to respiratory function; and 0.4–1.6 Hz, reflecting heart beat frequency (10). Wavelet analysis was performed using the wavelet toolbox in Matlab (7.8.0.347; The Mathworks, Inc., Natick, MA, USA) as described by Newman et al. (17). The relative amplitude was extracted for each of the five frequency bands (the average amplitude within a band divided by the average amplitude of the entire spectrum). This normalized amplitude takes into account the variation in the LDF signal strength between subjects (10,18,19). See supplemental figure 1 for examples of LDF recordings and non-normalized and normalized wavelet analyses.

Statistical analysis

All variables were first checked for normality of distribution. Data are presented as mean ± SD or median with range when applicable. The relations between BMI, microvascular vasomotion domains, adiponectin and leptin were assessed using univariable linear regression analyses. Next, multivariable linear regression analyses were performed to investigate whether the association between BMI and microvascular vasomotion domains remained when adjusting for adiponectin. For graphic representation, we re-analysed significant relationships using a restricted cubic spline function. These are piecewise polynomial functions that are constrained to join smoothly at points called knots. They provide insight in potentially non-linear relationships without the need to transform independent variables into categorical variables. All data points are thus used to estimate the outcome value at each level of independent variable, as opposed to categorical variables, which assume a constant risk within categories. Testing for non-linearity in the linear regression models was carried out using likelihood ratio (score) tests. All spline regression analyses were performed using R (version 2.15.2; The R Foundation for Statistical Computing, Vienna, Austria) (20). All analyses were adjusted for sex. A two-tailed P-value < 0.05 was considered

significant. All analyses other than spline regression were performed using the statistical software package SPSS (version 18.0, SPSS, Inc., Chicago, Il, USA).

Results

Characteristics of the study group

Baseline characteristics are shown in table 1. As expected, mean adiponectin was higher in women than men (12.1 ± 3.6 mg/L vs. 7.7 ± 2.5 mg/L, student t-test $p < 0.0001$).

BMI and adiponectin are associated with the neurogenic domain of microvascular vasomotion

Univariable regression analyses between BMI, adiponectin and vasomotion are displayed in Table 2. As expected, BMI was inversely related to adiponectin (β -0.18, $p = 0.028$) and positively related to leptin (β 2.22, $p < 0.0001$). BMI also showed an inverse relation with the normalized amplitude of the neurogenic vasomotion domain (β -0.011, $p = 0.046$). BMI was also positively associated with the normalized amplitude of the cardiac domain (β 0.003, $p = 0.020$). There was no significant association between BMI and the normalized amplitude of the endothelial domain (b 0.013, $p = 0.17$, stratified for gender: men β 0.008, $p = 0.62$, women β 0.15, $p = 0.21$). Only including subjects with a minimum LDF signal length of 16.7 min (i.e. 10 'endothelial contractions' or more, $n = 64$) did not affect the results (data not shown). In addition, adiponectin showed a positive relation with the normalized amplitude of the neurogenic vasomotion domain (β 0.016, $p = 0.019$). Leptin showed no significant relations with vasomotion (table 2). The other measures of adiposity such as trunk fat mass as assessed by dual-energy X-ray absorptiometry and the sum of four skin folds (21) showed directionally similar, but less significant results compared to BMI (supplemental table 1).

Adiponectin partly explains the association between adiposity and the normalized amplitude of the neurogenic domain of the vasomotion spectrum Because adiponectin levels were significantly higher in women in the AGHLS cohort, we investigated whether the criteria for effect modification were met for sex when using a regression model with adiponectin and the normalized amplitude of the neurogenic domain. This was not the case ($p = 0.62$ for interaction term). Subsequently, we investigated whether the association between BMI and the normalized amplitude of the neurogenic domain could statistically be explained, completely or at least partly, by lower adiponectin. An increase in BMI of 1 kg/m2 was associated with a decrease in relative amplitude of 0.011 in the neurogenic domain (95% CI: -0.022 to -0.0003; $p = 0.046$). After adjustment for adiponectin, the regression coefficient of the association between BMI and the neurogenic domain of the vasomotion spectrum decreased by 18% (b -0.009, 95% CI: -0.020 to 0.002; $p = 0.125$) and was no longer statistically significant (table 2). Adjustment for adiponectin did not affect the association between BMI and the cardiac vasomotion domain. Spline regression analyses showed the relationship between BMI and the normalized amplitude of the neurogenic domain to be linear throughout the entire BMI spectrum (figure 1). This linear relationship remained after adjustment for adiponectin (Score test; $p = 0.42$).

Discussion

Vasomotion amplitudes are known to differ between extreme adiposity phenotypes (11,12). Our study shows a linear relationship between BMI within the population range and the normalized amplitude of the neurogenic vasomotion domain. Both parameters were in turn associated with adiponectin. Furthermore, plasma adiponectin, at least statistically, explained part of the association between BMI and the normalized amplitude of the neurogenic domain. Of the three frequency domains in the vasomotion spectrum thought to contribute to local microvascular flow in the postulated physiological framework, that is endothelial, neurogenic and myogenic, the normalized amplitude of the neurogenic domain showed consistent relationships with BMI and adiponectin.

The 0.02 to 0.06-Hz frequency domain has been attributed to neurogenic activity after studies using local or ganglionic nerve blockade and sympathectomy (22,23). As the sympathetic nervous system (SNS) has been regarded as the source of the neurogenic domain in the vasomotion signal and SNS activity is thought to be increased in obesity (24), one could expect a higher amplitude in the neurogenic domain, that is more sympathetic rhythmic contractility in overweight/obese subjects (as is suggested by the higher normalized amplitude of the cardiac domain). Nevertheless, Agapitov et al. demonstrated a dissociation between increased SNS activity (microneurographic sympathetic nerve activity to skeletal muscle) and normal sympathetic vascular tone (as assessed via adrenergic receptor blockade by phentolamine) in normotensive obese individuals (25). These observations are consistent with the lower normalized amplitude in the normotensive overweight/obese subjects in this study and in line with earlier work by our group and others (11,12). A lower amplitude of the neurogenic domain (in other words a loss of sympathetic contractility) or vasomotion in general could be regarded as a shift towards pathology. The loss of randomness in precapillary arteriolar contractility signifies a decrease in adaptive response to changes in metabolic demand (26). Indeed, in a recent study in a different group of subjects, a decrease in the normalized amplitude of the neurogenic domain during a hyperinsulinemic euglycemic clamp was associated with lower insulin-augmented capillary recruitment and lower insulin-mediated glucose uptake (27/chapter 2). This supports the role for vasomotion, and the neurogenic domain in particular, in controlling blood flow distribution in skeletal muscle, subsequent insulin-mediated glucose uptake and obesity related defects therein. Indeed, vasomotion/flowmotion was recently reported in skeletal muscle using contrast enhanced ultrasonography (28). We could not demonstrate a significant association between BMI and the normalized amplitude of the endothelial domain. Although this was expected after earlier work from our group, this earlier study included only female participants, making those results less generalizable (11). Indeed, when stratified for gender, this association was twice as strong in women as in men in the AGHLS cohort. Furthermore, in the previous study, the number of women was twice that of the AGHLS cohort and they were selected to show extreme differences in BMI (11). Adipokines such as adiponectin and leptin are thought to be important intermediates in the pathways linking obesity with vascular and metabolic insulin resistance (3). Adiponectin in particular has received wide attention showing, amongst others, insulin-sensitizing effects (4,5). We found that in the AGHLS cohort, adiponectin was associated with the normalized amplitude of the neurogenic domain. Adiponectin could, at least partly, explain the association between BMI and the neurogenic domain of vasomotion, suggesting a modulating role for adiponectin in vasoreactivity. Such modulating effects by the globular domain of adiponectin have been found in vivo (29). How adiponectin would exert this modulating effect is not yet clear. Whether it entails direct local effects on vasomotor tone (via systemic circulating adiponectin or adiponectin excreted by local (e.g. perivascular) adipose tissue (30)) or central (nervous system) effects (31,32,33) remains to be elucidated. Leptin, although strongly related to body fatness, did not show an association with any of the vasomotion domains. Although vasoactive effects of leptin have been described ex vivo (34,35), such results have not been reproduced in vivo (36,37). In addition, the role of other adipokines besides adiponectin and leptin should not be neglected. This study has several strengths and limitations. To the best of our knowledge, this is the first study (experimental or clinical) to assess possible relationships between adipokines and vasomotion. The AGHLS cohort is the largest cohort in which vasomotion has been studied to date, and is population based. Also, the large number of subjects enables elaborate multivariable analyses. Recently, we have referred to a general rule-of-thumb for vasomotion analyses requiring a minimum recording of 10 successive arteriolar contractions in a frequency domain to perform robust spectral analyses for that particular domain (27). For the current retrospective cohort study, we included 30 subjects with theoretically only nine contractions (based on the predefined bottom end of the frequency spectrum, i.e. 0.01 Hz). Although the increase in the number of subjects available for analyses greatly improves the overall power of the study and re-analysis using only the 64 subjects with 10 contractions or more did not affect the results, the inclusion of LDF signal lengths < 16.7 min (with a minimum of

15 min) might have added slightly to the non-significant results for the endothelial domain. On the other hand, because of its size and the numerous measurements already involved, the AGHLS cohort does not allow for laborious studies or invasive interventions such as the gold standard for whole-body insulin sensitivity, the hyperinsulinemic euglycemic clamp. Also, statistical modeling can only provide indirect evidence of casual pathways, and causality can only be shown in different study designs. Finally, we did not address the role of other adipokines than full-length adiponectin and leptin, such as adiponectin's globular domain, although this would probably have a small impact as associations between the adiponectin subforms in epidemiological studies are only moderately weaker for total adiponectin than for more selective measurements of active fractions (38).

In conclusion, our data support a linear association between increasing BMI and loss of neurogenic microvascular vasomotion, and suggest that adiponectin is a causal intermediate in this association. Further studies should be carried out to confirm our findings, address alternative causal pathways and determine the downstream effects of altered microvascular vasomotion on vascular and metabolic sequelae of increasing adiposity.

Tables

table 1 - characteristics of the study population

characteristic	mean±SD or median (range)
n (males)	94 (46)
age, y	42.1 ± 0.7
body mass index, $kg \cdot m^{-2}$	25.5 ± 3.8
waist, cm	85.4 ± 11.1
trunk fatness by DEXA, kg	10.1 ± 4.1
S4SF, mm	67.8 ± 24.1
systolic blood pressure, mm Hg	117 ± 15
diastolic blood pressure, mm Hg	71 ± 8
fasting plasma glucose, mmol/L	5.0 ± 0.5
adiponectin, mg/L	9.9 ± 3.8
leptin, ug/L	8.2 (0.5 - 83.4)
total cholesterol, mmol/L	5.0 ± 0.8
HDL-cholesterol, mmol/L	1.7 ± 0.4
LDL-cholesterol, mmol/L	2.8 ± 0.8
triglycerides, mmol/L	1.2 ± 1.0
smoker current, n (%)	14 (15)
smoker ever, n (%)	26 (28)
medication use, %	10 (9.4)
metabolic syndrome > 3 criteria by IDF, n (%)	8 (9)
Vasomotion frequency domains, normalized to total amplitude	
cardiac	0.17 ± 0.05
respiratory	0.28 ± 0.08
myogenic	0.86 ± 0.19
neurogenic	1.71 ± 0.21
endothelial	2.66 ± 0.34

Data are presented as mean ± SD or median with interquartile range. S4SF indicates sum of four skinfold thicknesses. HOMA-R, homeostasis model assessment insulin resistance. IDF, International Diabetes Federation (Alberti KG, Zimmet P, Shaw J. Metabolic syndrome – a new worldwide definition. A Consensus Statement from the International Diabetes Federation. Diabet. Med. 23: 469-480, 2006). Cardiac indicates frequency spectrum attributed to heart beat, respiratory indicates frequency spectrum attributed to respiration, myogenic indicates frequency spectrum attributed to vascular smooth muscle cells, neurogenic indicates frequency spectrum attributed to SNS activity and endothelial indicates frequency spectrum attributed to endothelial activity. *Drug use was recorded in categories and not further specified. ‘Cardiovascular’ includes ‘antihypertensive’ and ‘antiarrhythmyc’.

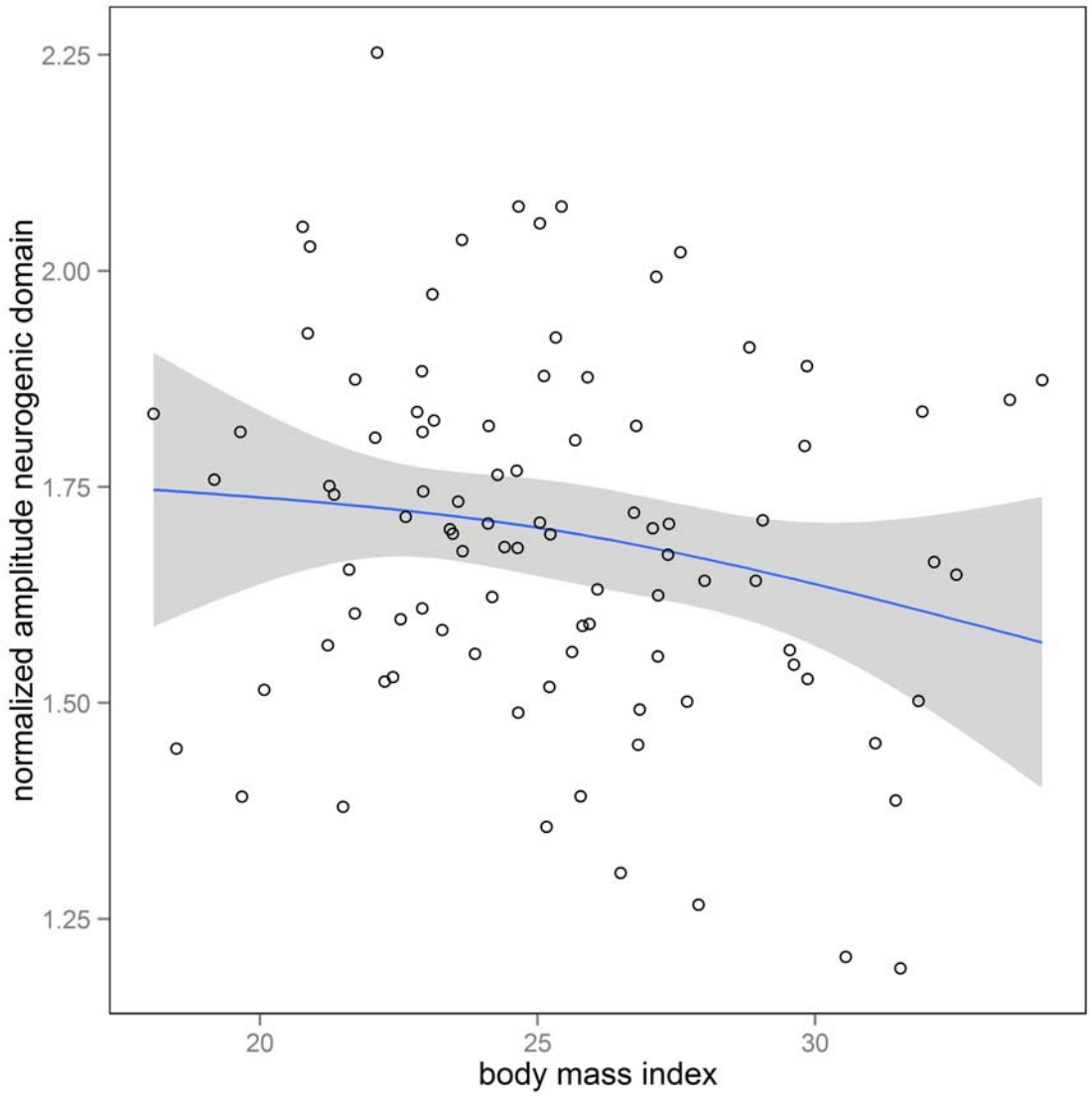
table 2 - regression analysis of normalized vasomotion domains

	BMI			ADN			LEP		
	β	95% CI	p	β	95% CI	p	β	95% CI	p
Model 1. adjusted for gender									
adiponectin	-0.18	-0.35- -0.02	0.028*	-	-	-	-	-	-
cardiac	0.003	0.001-0.006	0.021*	-0.003	-0.007-0.001	0.10	0.0003	-0.001-0.001	0.47
respiratory	0.001	-0.003-0.006	0.54	-0.003	-0.008-0.002	0.26	-0.0003	-0.002-0.001	0.67
myogenic	-0.004	-.015-0.007	0.50	-0.009	-0.022-0.005	0.20	-0.001	-0.005-0.002	0.38
neurogenic	-0.011	-0.022-0.000	0.046*	0.016	0.003-0.030	0.019*	-0.0002	-0.004-0.003	0.91
endothelial	0.013	-0.006-0.031	0.17	-0.004	-0.026-0.019	0.76	0.002	-0.004-0.007	0.54
Model 2. adjusted for gender and adiponectin									
adiponectin	-	-	-	-	-	-	-	-	-
cardiac	0.003	0.000-0.006	0.047*	-	-	-	-	-	-
respiratory	0.001	-0.004-0.005	0.73	-	-	-	-	-	-
myogenic	-0.006	-0.17-0.006	0.32	-	-	-	-	-	-
neurogenic	-0.009	-0.020-0.002	0.13	-	-	-	-	-	-
endothelial	0.013	-0.006-0.032	0.18	-	-	-	-	-	-

BMI and adipokines in 94 subjects. All models are adjusted for gender β = regression coefficient, 95% CI = 95% confidence interval, and p indicates the significance level of the relationship. *Significant association ($p < 0.05$). Cardiac indicates frequency spectrum attributed to heart beat, respiratory indicates frequency spectrum attributed to respiration, myogenic indicates frequency spectrum attributed to vascular smooth muscle cells, neurogenic indicates frequency spectrum attributed to SNS activity and endothelial indicates frequency spectrum attributed to endothelial activity.

Figures

figure 1



Relationship between BMI and the normalized amplitude of the neurogenic vasomotion domain in a spline regression model. The light band represents the 95% confidence interval.

References

1 Finucane MM, Stevens GA, Cowan MJ, Danaei G, Lin JK, Paciorek CJ, et al. National, regional, and global trends in body-mass index since 1980: systematic analysis of health examination surveys and epidemiological studies with 960 country-years and 9.1 million participants. Lancet 377: 557-567, 2011.

2 Sowers JR. Obesity as a cardiovascular risk factor. Am J Med 115(Suppl 8A): 37S-41S, 2003.

3 Scherer PE. Adipose tissue: from lipid storage compartment to endocrine organ. Diabetes 55: 1537-1545, 2006.

4 Yamauchi T, Kamon J, Waki H, Terauchi Y, Kubota N, Hara K, et al. The fat-derived hormone adiponectin reverses insulin resistance associated with both lipoatrophy and obesity. Nat Med. 8: 941-946, 2001.

5 Weyer C, Funahashi T, Tanaka S, Hotta K, Matsuzawa Y, Pratley RE, et al. Hypoadiponectinemia in Obesity and Type 2 Diabetes: Close Association with Insulin Resistance and Hyperinsulinemia. J Clin Endocrinol Metab. 86: 1930-1935, 2001.

6 de Boer MP, Meijer RI, Wijnstok NJ, Jonk AM, Houben AJ, Stehouwer CD, et al. Microvascular Dysfunction: A Potential Mechanism in the Pathogenesis of Obesity-associated Insulin Resistance and Hypertension. Microcirculation 19: 5-18, 2012.

7 Serné EH, Stehouwer CD, ter Maaten JC, ter Wee PM, Rauwerda JA, Donker AJ, et al. Microvascular function relates to insulin sensitivity and blood pressure in normal subjects. Circulation 99: 896-902, 1999.

8. Rücker M, Strobel O, Vollmar B, Roesken F, Menger MD. Vasomotion in critically perfused muscle protects adjacent tissues from capillary perfusion failure. Am J Physiol Heart Circ Physiol. 279: H550-H558, 2000.

9. Kvernmo HD, Stefanovska A, Kirkeboen KA, Kvernebo K. Oscillations in the human cutaneous blood perfusion signal modified by endothelium-dependent and endothelium-independent vasodilators. Microvasc Res. 57: 298-309, 1999.

10. Stefanovska A, Bracic M, Kvernmo HD. Wavelet analysis of oscillations in the peripheral blood circulation measured by laser Doppler technique. IEEE Trans Biomed Eng. 46: 1230-1239, 1999.

11. de Jongh RT, Serné EH, IJzerman RG, Jørstad HT, Stehouwer CD. Impaired local microvascular vasodilatory effects of insulin and reduced skin microvascular vasomotion in obese women. Microvasc Res. 75: 256-262, 2008.

12. Rossi M, Nannipieri M, Anselmino M, Pesce M, Muscelli E, Santoro G, et al. Skin vasodilator function and vasomotion in patients with morbid obesity: effects of gastric bypass surgery. Obes Surg. 21: 87-94, 2011.

13. Wijnstok NJ, Hoekstra T, van Mechelen W, Kemper HC, Twisk JW. Cohort profile: The Amsterdam Growth and Health Longitudinal Study. Int J Epidemiol. 42: 422-429, 2012.

14. te Velde SJ, Twisk JW, van Mechelen W & Kemper HC. Birth weight, adult body composition, and subcutaneous fat distribution. Obesity Research 11: 202-208, 2003.

15 Weiner JS, Lourie JA. Human biology, a guide to field methods. IBP handbook no. 9. Oxford: Blackwell; 1969.

16. Andersen KK, Frystyk J, Wolthers OD, Heuck C, Flyvbjerg A. Gender differences of oligomers and total adiponectin during puberty: a cross-sectional study of 859 Danish school children. J Clin Endocrinol Metab. 92: 1857-1862, 2007.

17 Newman JM, Dwyer RM, St-Pierre P, Richards SM, Clark MG, Rattigan S. Decreased microvascular vasomotion and myogenic response in rat skeletal muscle in association with acute insulin resistance. J Physiol. 587: 2579-2588, 2009.

18 Bracic M, Stefanovska A. Wavelet-based analysis of human blood-flow dynamics. Bull Math Biol. 60: 919-935, 1998.

19 Graaff R, Morales F, Smit AJ, de Jong ED, de Mul FF, Rakhorst G. Normalization of vasomotion in laser Doppler perfusion monitoring. Conf Proc IEEE Eng Med Biol Soc. 4076-4079, 2007.

20 Steenland K, Deddens JA. A practical guide to dose response analyses and risk assessment in occupational epidemiology. Epidemiology 15: 63-70, 2004.

21 Ketel IJ, Volman MN, Seidell JC, Stehouwer CD, Twisk JW, Lambalk CB. Superiority of skinfold measurements and waist over waist-to-hip ratio for determination of body fat distribution in a population-based cohort of Caucasian Dutch adults. Eur J Endocrinol. 156: 655-661, 2007.

22 Kastrup J, Bülow J, Lassen NA. Vasomotion in human skin before and after local heating recorded with laser Doppler flowmetry. A method for induction of vasomotion. Int J Microcirc Clin Exp.8: 205-215, 1989.

23 Söderström T, Stefanovska A, Veber M, Svensson H. Involvement of sympathetic nerve activity in skin blood flow oscillations in humans. Am J Physiol Heart Circ Physiol. 284: H1638-H1646, 2003.

24 Snitker S, Macdonald I, Ravussin E, Astrup A. The sympathetic nervous system and obesity: role in aetiology and treatment. Obes Rev. 1: 5-15, 2000.

25 Agapitov AV, Correia ML, Sinkey CA, Haynes WG. Dissociation between sympathetic nerve traffic and sympathetically mediated vascular tone in normotensive human obesity. Hypertension 52: 687-695, 2008.

26 Goldberger AL, Amaral LA, Hausdorff JM, Ivanov PCh, Peng CK, Stanley HE. Fractal dynamics in physiology: alterations with disease and aging. Proc Natl Acad Sci USA 99(Suppl 1): 2466-2472, 2002.

27 de Boer MP, Meijer RI, Newman J, Stehouwer CD, Eringa EC, Smulders YM, Serné EH. Insulin-induced changes in microvascular vasomotion and capillary recruitment are associated in humans. Microcirculation 21: 380-387, 2014.

28 Rattigan S, Bradley E, Richards S, Keske M. Detection of microvascular flowmotion within skeletal muscle by real-time contrast-enhanced ultrasound (RT-CEU) and laser Doppler flowmetry (LDF). Diabetes 62(suppl 1): A509, 2013.

29 Zhao L, Chai W, Fu Z, Dong Z, Aylor KW, Barrett EJ, et al. Globular Adiponectin Enhances Muscle Insulin Action via Microvascular Recruitment and Increased Insulin Delivery. Circ Res. 112: 1263-1271, 2013.

30 Meijer RI, Bakker W, Alta CL, Sipkema P, Yudkin JS, Viollet B, et al. Perivascular Adipose Tissue Control of Insulin-Induced Vasoreactivity in Muscle Is Impaired in db/db Mice. Diabetes 62: 590-598, 2013.

31 Viollet B, Andreelli F, Jørgensen SB, Perrin C, Geloen A, Flamez D, et al. The AMP-activated protein kinase alpha2 catalytic subunit controls whole-body insulin sensitivity. J Clin Invest. 111: 91-98, 2003.

32 Hoyda TD, Samson WK, Ferguson AV. Adiponectin depolarizes parvocellular paraventricular nucleus neurons controlling neuroendocrine and autonomic function. Endocrinology 150: 832-840, 2009.

33 Spranger J, Verma S, Göhring I, Bobbert T, Seifert J, Sindler AL, et al. Adiponectin does not cross the blood-brain barrier but modifies cytokine expression of brain endothelial cells. Diabetes 55: 141-147, 2006.

34 Lembo G, Vecchione C, Fratta L, Marino G, Trimarco V, d'Amati G, et al. Leptin induces direct vasodilation through distinct endothelial mechanisms. Diabetes 49: 293-297, 2000.

35 Vecchione C, Aretini A, Maffei A, Marino G, Selvetella G, Poulet R, et al. Cooperation between insulin and leptin in the modulation of vascular tone. Hypertension 42: 166-170, 2003.

36 Jalali A, Morgan DA, Sivitz WI, Correia ML, Mark AL, Haynes WG. Does leptin cause functional peripheral sympatholysis? Am J Hypertens. 14: 615-618, 2001.

37 Mitchell JL, Morgan DA, Correia ML, Mark AL, Sivitz WI, Haynes WG. Does leptin stimulate nitric oxide to oppose the effects of sympathetic activation? Hypertension 38: 1081-1086, 2001.

38 Fisher FM, Trujillo ME, Hanif W, Barnett AH, McTernan PG, Scherer PE, et al. Serum high molecular weight complex of adiponectin correlates better with glucose tolerance than total serum adiponectin in Indo-Asian males. Diabetologia. 48: 1084-1087, 2005.

Supplemental material

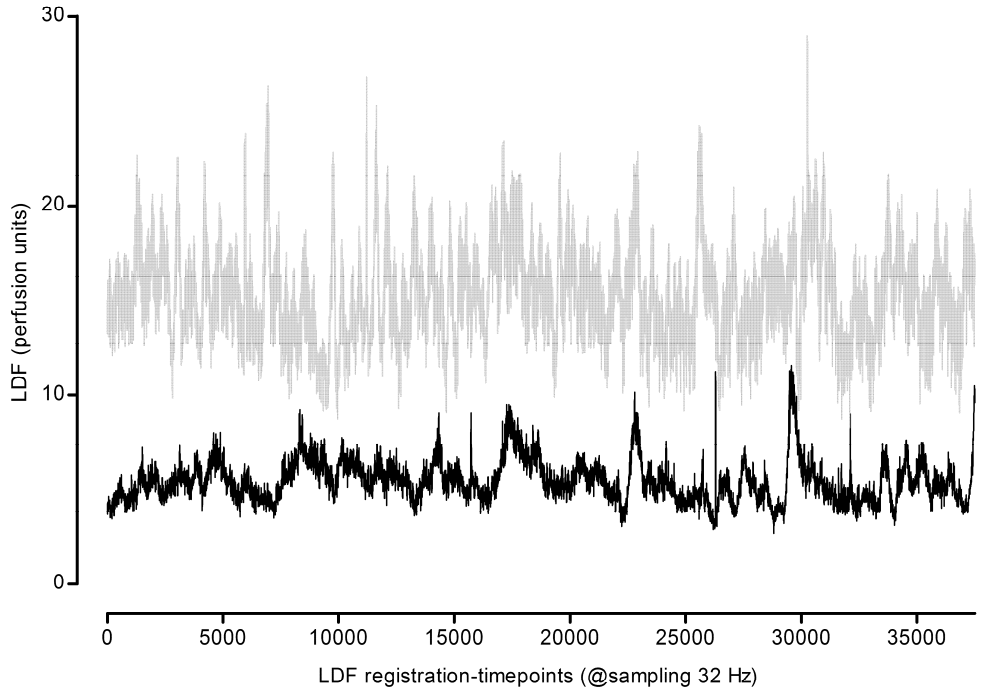
table 1 - regression analysis of normalized vasomotion domains

	dexa			S4SF		
	β	95% CI	p	β	95% CI	p
Model 1. adjusted for gender						
adiponectin	-0.17	-0.32- -0.18	0.029*	-0.024	-0.051-0.004	0.089
cardiac	0.002	-0.001-0.005	0.16	0.0002	-0.0003-0.001	0.37
respiratory	0.002	-0.002-0.006	0.41	0.0002	-0.0005-0.001	0.55
myogenic	-0.004	-0.14-0.007	0.48	-0.001	-0.002-0.001	0.54
neurogenic	-0.009	-0.19-0.002	0.11	-0.002	-0.003-0.0002	0.084
endothelial	0.11	-0.006-0.028	0.21	0.002	-0.001-0.005	0.12
Model 2. adjusted for gender and adiponectin						
adiponectin	-	-	-	-	-	-
cardiac	0.002	-0.001-0.004	0.29	0.0002	-0.0003-0.001	0.53
respiratory	0.001	-0.003-0.005	0.56	0.0001	-0.001-0.001	0.68
myogenic	-0.006	-0.16-0.005	0.30	-0.001	-0.003-0.001	0.39
neurogenic	-0.006	-0.016-0.004	0.25	-0.001	-0.003-0.001	0.17
endothelial	0.11	-0.007-0.028	0.23	0.002	-0.001-0.005	0.13

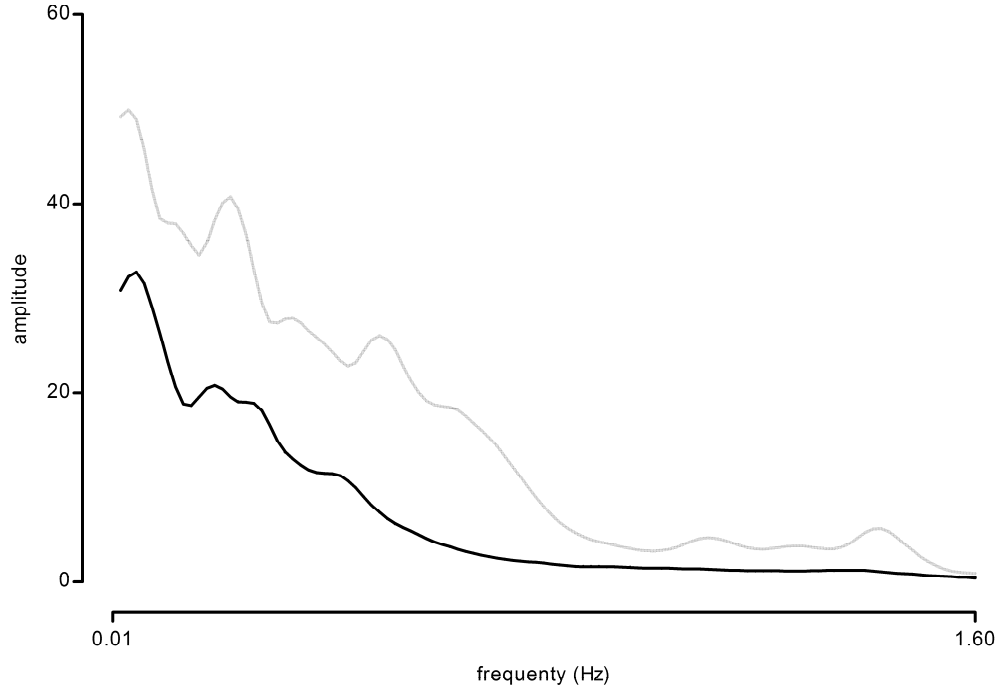
Trunk fat via DEXA, sum of 4 skin folds and adiponectin in 94 subjects. DEXA indicates trunk fat in kilograms as assessed via whole body dual-energy X-ray absorptiometry, S4SF indicates sum of 4 skinfold thicknesses, β = regression coefficient, 95% CI = 95% confidence interval, and p indicates the significance level of the relationship. *Significant association ($p < 0.05$). Cardiac indicates frequency attributed to heart beat, respiratory indicates frequency spectrum attributed to respiration, myogenic indicates frequency spectrum attributed to vascular smooth muscle cells, neurogenic indicates frequency spectrum attributed to SNS activity and endothelial indicates frequency spectrum attributed to endothelial activity.

figure 1

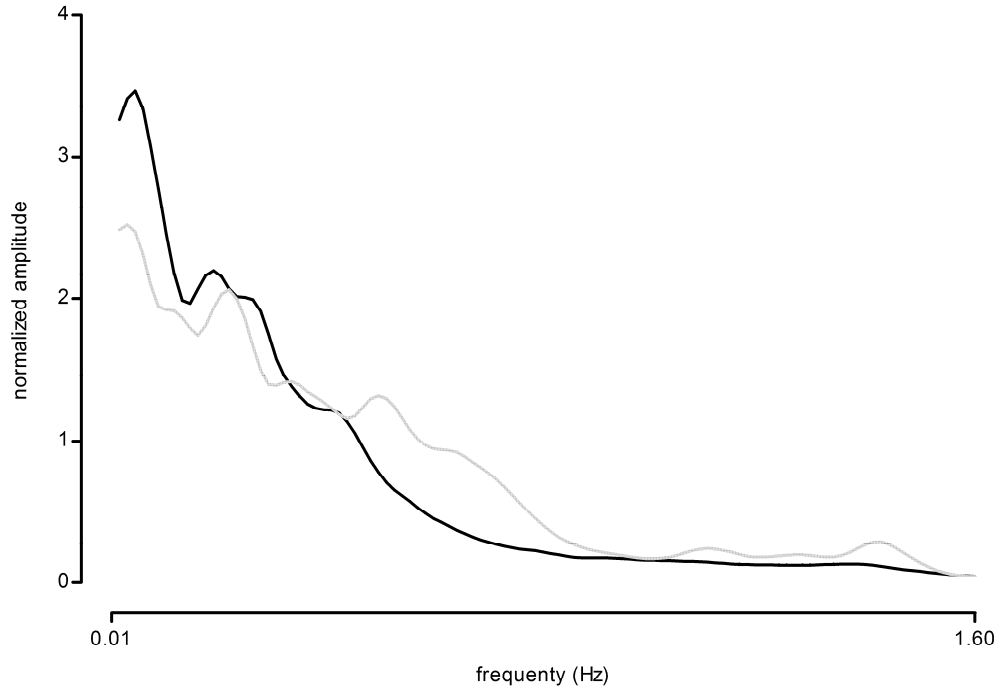
A


30
20
10
0
LDF (perfusion units)
0
5000
10000
15000
20000
25000
30000
35000
LDF registration-timepoints (@sampling 32 Hz)

B


60
40
20
0
amplitude
0.01
1.60
frequenty (Hz)

C



A. 2 examples of an LDF recording (see methods Laser Doppler Flowmetry). B. 2 examples of non-normalized results after wavelet analyses on LDF recordings from figure A (see methods Vasomotion). C. 2 examples of normalized results after wavelet analyses on LDF recordings from figure A (see methods Vasomotion).