6

The relationship between 30-year developmental patterns of body fat and body fat distribution and vascular properties: The Amsterdam Growth and Health Longitudinal Study

6

Nutrition and Diabetes: 2013: 1-7

Authors Nienke J. Wijnstok, Erik H. Serné, Trynke Hoekstra, Fleur Schouten, Yvo M. Smulders, Jos W. R. Twisk

Abstract

Introduction: Although body fat and body fat distribution are known to be related to CVD, it is unknown whether specific 30-year developmental patterns of body fat are associated with CVD. This study examines the existence of distinct developmental patterns of total fat measured by the sum of 4 skinfolds (S4SF) and body fat distribution measured by skinfolds ratio (SFratio) and relates these patterns to micro- and macrovascular function.
Methods: In 2006, 259 apparently healthy subjects were examined on micro- and macrovascular function, using video microscopy, and carotid ultrasound sonography. Body fat, using both S4SF and SFratio, was measured for 10 times over 30 years, from 13 years onwards. Latent class growth analyses (LCGA) was used to obtain distinct developmental patterns of S4SF and SFratio. This

is a data drive, hypothesis generating approach and could possibly give a new perspective on body fatness over time. Additionally, a mixed method approach is used to obtain individual growth parameters. Linear regression analyses were used to examine the relationship of these patterns and individual growth parameters with micro- and macrovascular function.
Results: LCGA identified normal and unfavourable developmental patterns in S4SF and SFratio. Both males and females with an unfavourable developmental pattern of S4SF showed impaired carotid compliance (β=-0.216, p=0.004; β=-0.109, p=0.039), carotid distensibility (β=-5.078, p=0.001; β=-5.118, p<0.001) and Young's Elastic Modulus (β=0.066, p=0.065; β=0.107, p<0.001). In contrast, no relationship for microvascular function with developmental patterns of S4SF was found. Developmental patterns of SFratio were associated with neither measures of micro- nor macrovascular function. No associations were using the individual growth parameters.
Conclusions: For macrovascular function there is a relationship of 30-year developmental patterns of S4SF. No such relationship was found 30-year developmental patterns of S4SF or SFratio with microvascular function.

Introduction

The epidemic proportions of obesity or high body fat are widely recognised[195]. One of the main negative effects of high body fat is the development of cardiovascular disease (CVD). Even in apparently healthy adults, high body fat, as well as a central pattern of body fat, is cross-sectionally associated with microvascular and macrovascular function[29,223]. Fat induced changes in microvascular function are hypothesized to affect insulin sensitivity and peripheral resistance, which may cause diabetes and hypertension[11,223]. On a macrovascular level, fat induced changes are hypothesized to affect local and regional vessel wall stiffness parameters which may adversely impact on atherosclerosis development and cardiac afterload. Both changes in micro- and macrovascular function are regarded as early markers of vascular dysfunction which can appear in relatively healthy subjects[11,223]. Despite several studies[26,194,198,199,202,205,224–228], the association, if any, between particular developmental patterns of body fat and vascular dysfunction is unknown.

A relatively new explorative method for identifying developmental patterns of for example body fat is latent class growth analyses[228]. To the best of our knowledge, it is unknown whether distinct developmental patterns of body fat are associated with micro and macrovascular perturbations. Therefore, in the current study, the presence of distinct 30 year developmental patterns (from 13 to 42 years of age) of body fat is examined. The main aim of the study is to examine the potential association of such distinct developmental patterns in relation with both micro- and macrovascular function.

Subjects and Methods

Study population

The observational Amsterdam Growth and Health Longitudinal Study (AGHLS) started in 1977 and initially consisted of approximately 600 boys and girls at the age of 13years[26]. Over the last 30 years, 10 follow-up visits have taken place in 1977, 1978, 1979, 1980, 1985, 1991, 1993, 1996, 2000 and 2006 respectively. These have resulted in the current database including anthropometric (body height, body weight and skinfold thickness) and biological parameters (serum sample parameters and blood pressure). The study is described elsewhere in detail26. In the most recent round of measurement, when subjects reached the age of 42 years, microvascular function and large artery properties were assessed in 344 subjects in addition to the regular measurements. The study was approved by the medical ethics committee of the VU University Medical Center and all subjects gave their written informed consent. Only participants who completed at least 3 measurement rounds were included, and participants using cardiovascular related medication (N=7) were excluded from further analyses.

Body fat and body fat distribution

In the AGHLS, body fat and body fat distribution are operationalized in different ways. Body Mass Index (BMI) was calculated with weight and height using standard equipment. The sum of four skinfold thickness (S4SF) was calculated by summing the thickness of the biceps, triceps, subscapular and suprailiac skinfold. The skinfold thickness ratio (SFratio) is used as indicator of body fat distribution and is defined as the subscapular plus the suprailiac skinfold divided by the biceps plus the triceps skinfold thickness. Central body fat solely (CentralSF) is defined as the subscapular and the suprailiac skinfold taken together. A holtain calliper was used (Holtain London, UK)[23].

Microvascular function

Nailfold capillary videomicroscopy (Capiscope®, KK technologies, Devon, UK) was used to measure microvascular function. Capillaries were visualised in the nailfold of the dorsal skin of the third finger with a system magnification of 100x. With this technique all capillaries that are erythrocyte perfused (capillary density) are visualised. For all subjects two separate fields of 1mm2 were recorded on videotapes. Per field, both baseline perfusion and peak reactive hyperaemia were measured. At first, *baseline capillary density* was visualised counting capillaries that were constantly perfused during 15seconds in resting state. With counting the maximal number of perfused capillaries after 4 minutes of arterial occlusion (300 mmHg), *peak reactive hyperaemia* was measured. Microvascular recruitment was calculated as peak reactive hyperaemia minus baseline perfusion. All measurements were separated with a 5 minute

rest-period and the capillary density was reported as the number of perfused capillaries/mm^2.

Restricting conditions to measure microvascular parameters in participants were a minimum hand temperature of 28°C, fasting state and at least 30 minutes rest before the measurement since this could possibly affect microvascular perfusion.

Reproducibility of the microvascular parameters were tested using intra-class correlation coefficients (ICC's). In our hands intra-observer ICC's of capillary density counts during baseline and after 4 minutes of arterial occlusion were .97 and .96 and inter- observer ICC's were .86 and .90 respectively.

Macrovascular function

Macrovascular function was obtained with ultrasound sonography. The Ultrasound scanner (Wall track system 2; Pie Medical, Maastricht, Netherlands) was used to measure carotid, and femoral artery capacities. The standardized procedures to obtain artery capacities, for the estimation macrovascular function are described elsewhere in detail[57]. Carotid diameter (D), distension (ΔD), Intima Media Thickness (IMT) and carotid and femoral pulse wave transit (TT). Also a calculated score was made for distensibility (DC; $(2\Delta D x D + 2\Delta D^2)$), compliance (CC; $(\pi x(2D x \Delta D + \Delta D^2))$ and the Youngs Elastic Modulus (YEM; D/(IMTxDC). The DC reflects elastic properties and the CC reflects a buffering capacity given at a local pulse pressure. These are both local parameters for which account, the higher the value the more stiff arteries are. YEM represents local arterial elasticity. Note that the higher the YEM, the less elastic the artery. Furthermore, using both carotid and femoral capacities, pulse wave velocity (PWV) as a regional estimate of arterial stiffness, is calculated as carotid-femoral distance /carotid to femoral TT.

Reproducibility of macrovascular parameters was tested in 2006[57]. In our hands inter-observer Coefficients of Variation (CV) were 2.7% for the carotid, and 4.9% for femoral diameter properties respectively. For distension properties CV's were 9.5% for carotid, and 28.3% for femoral properties. For carotid IMT, a CV of 6.2% was found.

Covariates

Systolic blood pressure (SBP) was measured at 5 minute intervals, during 60 minutes, in a supine position with an automated device (Dinamap Procare 100, GE Healthcare, Germany). Several fasting blood parameters, such as glucose, insulin, high density lipoprotein cholesterol (HDL) and triglyceride (TG) levels were measured by enzymatic or hexokinase techniques (Roche Diagnostics, Mannheim, Germany). HOMA-IR as a measure of insulin sensitivity was calculated as Glucose (mmol/L) * insulin (mU/L) / 22.5.

Retrospective information on known family history of diabetes and cardiovascular events, medication use and smoking were obtained with validated questionnaires.

Statistical analyses

Statistical analyses were divided into two steps. In the first step, latent class growth analyses (LCGA) was performed for the four indicators of body fat: S4SF, SFratio and BMI. The aim of LCGA is to model heterogeneity by identifying an unspecified number of groups on the basis of a similar developmental pattern[213,228,229]. A piecewise model is computed, using a linear intercept and slope over time, and allowing for different phases in development, (i.e. additive unique intercept and slope over the three phases) in adolescence (1977-1980), 20's (1985-1993) and 30's (1996-2006). Time differences between the rounds of measurement are taken into account. The model is considered optimal when individuals are most alike within one subgroup, and most different between subgroups. To determine the optimal number of groups, a "forward" approach was taken, starting with a model with one group, implying that all individuals in the study had the same developmental pattern. Subsequently, one group at a time was added, and the model fit was assessed by the Bayesian Information Criterion (BIC)[230]. After each step of adding a new group, the model fit was considered better if the BIC decreased. The final number of groups was derived not only on the basis of model fit, but also on clinically relevant differences between the groups. Thus, if addition of a new group led to a model with clinically indistinguishable developmental pattern, the model was not considered improved[230]. The LCGA analyses were conducted with Mplus 5.21[231]. In the second step, linear regression analyses were performed, in which the relationship between class membership and vascular function was examined. After a crude model, which depicts the mean difference between the groups and indicates significance of this difference, multivariate analyses allows for examination of potential mediators such as SBP, HDL, TG and HOMA-IR. Further adjustment was made for the potential confounders smoking, and retrospective information on known family history of diabetes and cardiovascular events.

Additionally, the relationship between the individual growth parameters and the micro- and macrovascular outcomes were analysed with a cross-sectional linear regression analysis. To obtain these individual growth parameters a mixed model analysis was performed, in which a linear function with time was used to analyse the development of body fat indicators over time and in which both a random intercept and random slope were added to the model. From this linear development two individual growth parameters were obtained, i.e. the mean value (or intercept) and the linear slope over time. The mixed models analyses were performed in MLwiN 2.27 (Centre for Multilevel Modelling, Bristol, UK).

Linear regression analyses were performed in PASW statistics 18.0. All analyses were performed separately for males and females.

Results

Table 11 shows descriptive information of the variables used in the present study and Figure 8 shows the distinct developmental patterns of A: S4SF and B: SFratio. Central body fat solely is highly correlated (r = 0.96 for males and r = 0.94 for females) with total body fatness and therefore not analysed separately. Based on model fit (table 12) indicators and clinical relevance, the final model for both S4SF and SFratio were 2-class solutions, for both males and females. For these models, the developmental pattern that was most alike 'normal' growth as observed in previous studies[194,198,199,202,205,224–227,] was appointed as favourable (solid lines), and the distinct developmental pattern as unfavourable (dashed lines). For BMI, a 1-class solution was found to be the best. A 2-class model for BMI lead to overlapping developmental patterns, a 3-class model lead to very small and partly overlapping developmental patterns with only 6 males and 8 females in the added class.

Tables 13 (males) and 4 (females) show the results of the linear regression analyses to examine the relationship between class membership (independent) and vascular function (dependent). In crude analysis for both males and females carotid compliance and carotid distensibility were significantly different between the two S4SF patterns. In females only, YEM differed significant between the two developmental patterns of S4SF, while IMT differed significant between the two developmental patterns of SFratio. For all relationships as found in the current study, subjects in the clinically less favourable pattern showed unfavourable macrovascular function. However, examining potential mediators attenuated the relationships in such a way that they were not significant anymore. SBP was on average the most important mediator explaining on average 49% and 45% of the relationships between class membership and macrovascular function in males and females respectively. HOMA-IR was the next important mediator explaining on average 14% and 25% percent of these relationships for males and females. For example, the difference between the univariate analysis examining carotid distensibility and class membership in females shows a β of -5.118 whereas the multivariate model for this relationship shows a β of -2.647 which is almost 50% difference. Therefore, biological risk factors, and most pronounced SBP and HOMA-IR, can partly explain the relationship, as presented in table 13 and 14, between class membership and macrovascular function. Further adjustment for smoking and retrospective information on known family history of CVD and diabetes did not alter these relationships (data not shown).

For microvascular function, no significant relationship was found with developmental patterns of either S4SF or SFratio.

Table 11. Descriptive information of the AGHLS population.

Measures in 2006	Males (N =116) Mean±SD	Females (N =143) Mean±SD
Body fat		
BMI (kg/cm^2)	25.2 ± 2.9	23.8 ± 3.3
S4SF (in mm)	539 ± 17.4	697 ± 19.5
SFratio (subscapular + suprailiac/biceps + triceps)	0.69 ± 0.07	0.56 ± 0.07
Central SF (subscapular + suprailiac	374 ± 142	388 ± 151
Microvascular function		
Baseline capillary density (capillaries per mm^2)	40.8 ± 15.9	41.2 ± 14.6
Peak reactive hyperaemia (capillaries per mm^2)	74.9 ± 21.5	82.2 ± 22.1
Recruitment (PRH minus baseline)	34.1 ± 12.8	40.9 ± 15.0
Macrovascular function		
Carotid distensibility (10^{-3}/kPa)	25.2 ± 7.1	26.2 ± 6.9
Carotid compliance (mm^2/kPa)	1.09 ± 0.34	0.95 ± 0.28
Pulse wave velocity ((m/s)^2)	8.59 ± 1.76	8.08 ± 1.48
Carotid Young's elastic modulus (10^{-3}/kPa)	0.50 ± 0.16	0.43 ± 0.14
Carotid Intima Media Thickness (mm)	0.66 ± 0.12	0.66 ± 0.12
CVD risk parameters		
Systolic Blood Pressure (mmHg)	122.5 ± 12.74	110.4 ± 12.86
HDL cholesterol (mmol/L)	1.5 ± 0.34	1.9 ± 0.39
Triglycerides (mmol/L)	1.3 ± 0.78	1.0 ± 0.45
HOMA-IR (Glucose (mmol/L) * insulin (mU/L) / 22.5)	1.67 ± 0.97	1.45 ± 0.65
Retrospective questionnaires		
Known family history of diabetes (Yes%)	3.4%	2.1%
Known family history of CVD (Yes%)	56.9%	69.9%
Smoking (Yes%)	16.4 %	13.3%

Table 12. Model fit indices (BIC) for gender specific models of body fat.

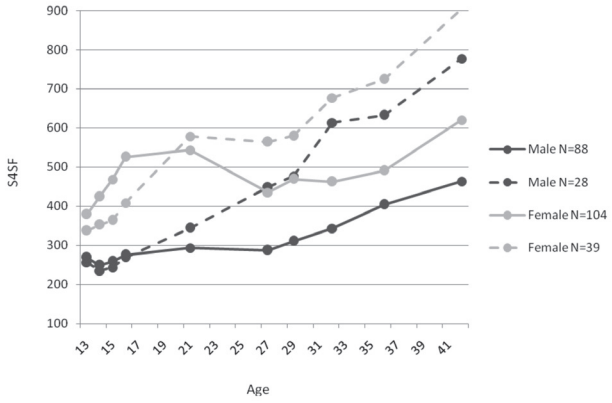
	BMI	S4SF	SFratio
1 class	6681	20207	10141
2 class	6265 NCD	19872	10033
3 class	6147 NCD	DNC	10047

DNC= Did not converge

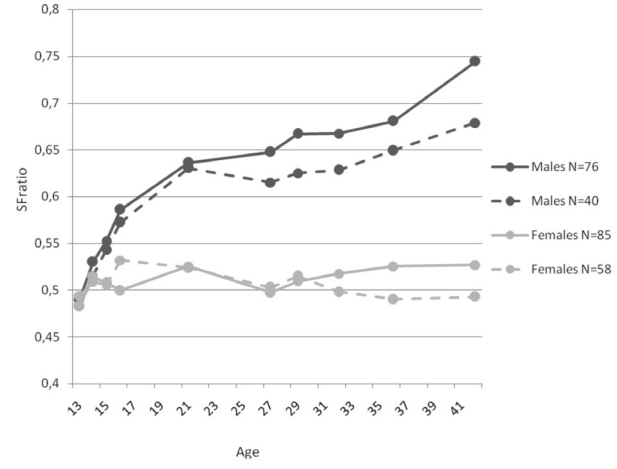
NCD= Not clinically distinct (i.e. very small or overlapping groups)

Figure 8. Gender specific developmental patterns of body fat over time in the AGHLS.

A.S4SF: Gender specific two class model.



B: SFratio: Gender specific two class model.



The dark-grey lines represent the boys and the light-grey lines represent the girls.
Solid lines represent the normal developmental patterns and the dashed lines have an unfavourable developmental pattern.

Table 13. Results of linear regression analyses regarding body fat and vascular function for males.

	Model 1 (crude)		Model 2 (adjusted for biological risk factors)	
	S4SF β (CI)	SFratio β (CI)	S4SF β (CI)	SFratio β (CI)
Microvascular function				
Recruitment	0.625 (-4.892 to 6.143)	-0.189 (-5.158 to 4.779)	0.683 (-5.888 to 7.255)	-0.287 (-5.614 to 5.039)
Macrovascular function				
Carotid distensibility	-5.078 ** (-8.039 to -2.118)	0.759 (-2.049 to 3.566)	-2.194 (-5.108 to 0.720)	1.622 (-0.753 to 3.998)
Carotid compliance	-0.216 ** (-0.361 to -0.070)	0.037 (-0.100 to 0.173)	-0.140 (-0.296 to 0.015)	0.062 (-0.066 to 0.190)
Pulse wave velocity	0.325 (-0.436 to 1.085)	0.092 (-0.606 to 0.789)	-0.065 (-0.941 to 0.812)	0.078 (-0.635 to 0.791)
Carotid Young's elastic modulus	0.066 (-0.004 to 0.136)	-0.006 (-0.071 to 0.058)	0.007 (-0.068 to 0.082)	-0.026 (-0.088 to0.035)
Carotid Intima Media Thickness	0.026 (-0.027 to 0.079)	-0.002 (-0.051 to 0.046)	< 0.000 (-0.063 to 0.064)	-0.006 (-0.059 to 0.046)

The significance level is indicated by * for p≤0.05 and ** for p≤0.01

Model 1 is the crude model, Model 2 is adjusted for systolic blood pressure, HDL-cholesterol, triglycerides and HOMA-IR

Table 14. Results of linear regression analyses regarding body fat and vascular function for females.

	Model 1 (crude)		Model 2 (adjusted for biological risk factors)	
	S4SF β (CI)	SFratio β (CI)	S4SF β (CI)	SFratio β (CI)
Microvascular function				
Recruitment	2.297 (-2.606 to 8.551)	-1.776 (-6.848 to 3.295)	2.811 (-4.196 to 9.818)	-1.656 (-7.127 to 3.815)
Macrovascular function				
Carotid distensibility	-5.118** (-7.592 to -2.645)	-0.483 (-2.850 to 1.883)	-2.256 (-4.876 to 0.365)	-0.647 (-2.694 to 1.399)
Carotid compliance	-0.109* (-0.213 to -0.006)	-0.029 (-0.124 to 0.067)	-0.009 (-0.129 to 0.111)	-0.040 (-0.133 to 0.052)
Pulse wave velocity	0.248 (-0.308 to 0.803)	-0.204 (-0.707 to 0.298)	-0.083 (-0.691 to 0.524)	-0.161 (-0.063 to 0.308)
Carotid Young's elastic modulus	0.107** (0.055 to 0.159)	0.032 (-0.017 to 0.081)	0.058* (<0.000 to 0.116)	0.034 (-0.011 to 0.079)
Carotid Intima Media Thickness	0.022 (-0.021 to 0.065)	-0.046* (-0.085 to -0.008)	0.016 (-0.035 to 0.067)	-0.038* (-0.077 to 0.001)

The significance level is indicated by * for p≤0.05 and ** for p≤0.01

Model 1 is the crude model, Model 2 is adjusted for systolic blood pressure, HDL-cholesterol, triglycerides and HOMA-IR.

Table 15. Results of linear regression using individual growth parameters.

	Males		Females	
	S4SF β (CI)	**SFratio β (CI)**	**S4SF β (CI)**	**SFratio β (CI)**
Microvascular function				
Recruitment				
Slope	13.85 (-10.63 – 38.32)	145.34 (-257.64 – 548.30)	15.28 (-1.71 – 32.28)	-202.50 (-1040.5 – 635.5)
Average	-3.50 (-8.64 – 1.63)	-61.32 (-162.98 – 4.34)	-0.66 (-3.10 – 1.78)	-42.82 (-103.30 –17.65)
Macrovascular function				
Carotid Distensibility				
Slope	-20.19 (-32.90 - -7.49)	-16.31 (-253.51– 220.90)	-4.22 (-11.67 – 3.23)	3.18 (-389.67 – 396.04)
Average	1.41 (-1.26 – 4.08)	-26.72 (-63.61 – 10.17)	-1.68 (-2.75 – -0.61)	-0.15 (-28.58 – 28.28)
Carotid Compliance				
Slope	-1.04 (-1.67 – -0.41)	-3.91 (-7.63 – 15.44)	0.03 (0.28 – 0.34)	0.13 (-15.67 – 15.92)
Average	0.13 (0.01 – 0.26)	-1.05 (-2.85 – 0.74)	-0.07 (-0.11 – -0.03)	-0.02 (-1.17 – 1.12)
Pulse wave velocity				
Slope	-3.15 (-6.48 – 0.18)	-27.08 (-86.25 – 32.08)	-1.31 (-3.00 – 0.36)	-75.63 (-157.45 – 6.19)
Average	0.83 (0.13 – 1.53)	-0.46 (-8.75 – 9.67)	0.23 (-0.01 -0.47)	6.57 (0.65 – 12.49)
Carotid Young's elastic modulus				
Slope	0.38 (-0.07 – 0.68)	0.64 (-4.87 – 6.15)	0.11 (-0.05 – 0.26)	4.78 (-3.39 – 112.94)
Average	-0.05 (0.11 – 0.02)	0.25 (-0.60 –1.10)	0.04 (0.02 – 0.06)	-0.20 (-0.80 – 0.39)
Carotid Intima Media Thickness				
Slope	0.04 (-0.28 – 0.20)	1.42(-2.60 – 5.45)	0.10 (-0.03 – 0.23)	-9.75 (-16.07 – 3.44)
Average	0.04 (-0.02 – 0.08)	0.12 (-0.52 – 0.77)	-0.02 (-0.04 – 0.01)	0.26 (-0.20 – 0.70)

The significance level is indicated by * for p≤0.05 and ** for p≤0.01; Skinfold parameter are presented in meters.

Table 15 shows the results of the analyses using individual growth parameters of S4SF and SFratio. These analyses show no significant relationship with either micro- or macrovascular function.

Discussion

In the present study we found that an unfavourable 30-year developmental pattern of total body fat was associated with macrovascular function, but not with microvascular function. The specific 30-year developmental patterns of body fat distribution on the other hand, were not associated with either macro- or microvascular function. We further found that the relationship between the development of body fatness and macrovascular function was highly mediated by SBP.

In the present study a relatively new statistical technique was used to classify subjects into different development patterns for both total body fat and body fat distribution. This is hypothesis generating technique

that identifies distinct developmental pattern over time, without a priori characteristics of this difference. It was shown that the two patterns found for total body fat start to differ from each other around the age of 20 years, while the two patterns for body fat distribution start to differ from each other around the age of 30 years. Furthermore, it was shown that although two different patterns were found for body fat distribution, the two patterns did not differ much from each other. That is probably also the reason why we did not find any relationship between body fat distribution with either micro- or macrovascular function except for IMT in females, although in the literature body fat distribution seems to be more important in relation to CVD risk compared to total body fat[9,57,232].

Comparing the currently used LCGA (table 13 and 14) with the more conventional approach using individual growth parameters shows some differences. Where no associations were observed with individual growth parameters, two distinct developmental patterns differed on macrovascular function. LCGA is a data driven approach that helps generating hypothesis. One of the advantages of using developmental trajectories is that these trajectories combine slope an average value in to one pattern, while the approach using individual growth parameters, the two are separated.

The association of total body fat with macrovascular function is attenuated after examining potential mediation for biological risk factors, by adding variables such as SBP and HOMA-IR, which on average account for about 14% and 45% of the explained variance in males and 25% and 49% in females. When both are added to the regression model, more than 50% of the relationships were explained. This was more or less expected because both SBP and HOMA-IR are known to be associated with macrovascular dysfunction[11,29,223].

In previous studies, body fat was found to be associated with both microvascular function[1,9,11,29] and macrovascular function[205,223,233]. The current study could only confirm the association with macrovascular function. Three possible explanations why, in the present study, macrovascular function was associated with body fat and microvascular function was not, may lie in the mechanism behind detrimental effects of body fat. First, the exchange of gases, nutrients, and metabolites between the blood and tissues occurs almost exclusively at the level of the capillaries, and adequate function of the capillaries, therefore, is essential for tissue and organ function. Given this important function of capillaries, it could be hypothesized that it may be important to protect capillary function. A protection mechanism of the capillaries that is alike, is described in normal physiological responses such as the venoarteriolar reflex, protecting the lower extremities from increased pressure after standing up from a sitting position or the myogenic response as response to increased resistance in arterioles, which all serve to protect the capillaries from severe increases in pressure, and which, in turn, could increase proximal blood

pressure[234,235]. In concordance with such a hypothesis, normal physiological responses, such as the prolonged hypertension, may be maintained. Thus, the luminal narrowing induced by the myogenic response may be maintained by a form of remodeling in which the vessel wall components are rearranged without growth, known as eutrophic inward remodeling[235,236]. Initially, this remodeling is considered to protect the capillaries from increased pressures, but later on it may be instrumental in causing microvascular rarefaction, due to increased systemic resistance, that subsequently reduces number of capillaries. In fact, the microcirculation, as an important site for pressure dissipation, is part of a vicious cycle that maintains and amplifies high blood pressure if it is not treated adequately[9,237]. The population in the present study has no such levels of blood pressure or increased peripheral resistance, and therefore, may not have entered this vicious circle. Second, microvascular function may be more influenced by perivascular adipose tissue than by systemic adipose tissue depots[183]. The methods to obtain estimates of body fat in the present study, are indicators of systemic adipose tissue, and therefore less related to microvascular function[183]. Third, and in addition to the previous explanation, the methods to measure central and peripheral body fat have some limitations. The currently used methods are well-known in epidemiological research, however these non-invasive measures cannot fully replace the idea of visceral and ectopic fat as identified as harmful in experimental research[183]. Using only non-invasive measure, it is simply impossible to separate the ectopic from the visceral part. Previous research using high central over peripheral fat, however has shown associations of these measures of body fat with CVD[11,29,57,195,223,224].

This study has some limitations. At first, the study population is an apparently healthy cohort, which means none of the subjects in this population have clinical levels of microvascular and macrovascular function[11,238]. Besides this, some of the microvascular parameters might not be influenced by the body fat levels within the healthy range, while more morbid levels of body fat could affect these parameters. Furthermore, measures as used are not gold standard, but were invented with the intention to non-invasively measure body fat parameters in population studies[23].

In conclusion, in healthy subjects, there was no relationship between life course developmental patterns of total body fat and body fat distribution and microvascular function. In contrast, there was a relationship between life course developmental patterns of total body fat and macrovascular function, which is largely explained by increased blood pressure and decreased insulin sensitivity. This relationship was not found for the developmental patterns of body fat distribution.