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ORIGINAL CONTRIBUTIONS

Discrepancies Between BMI and Classic Cardiovascular Risk Factors

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Abstract

Background Obesity is related to increased cardiovascular risk. It is unknown whether increasing levels of obesity also increase levels of cardiovascular risk factors and systemic inflammation. This study describes the relationship between classic cardiovascular risk factors and inflammatory markers with BMI in a group of obese and non-obese subjects.

Materials and Methods Obese subjects (BMI ≥ 30 kg/m^2; $n = 576$; mean $\pm$ SD BMI 43.8 ± 7.58 kg/m^2) scheduled for bariatric surgery were included. The reference population consisted of non-obese volunteers (BMI < 30 kg/m^2; $n = 377$, BMI 25.0 ± 2.81 kg/m^2). The relationship between BMI quintiles and the levels of cardiovascular risk factors was analyzed. Adipose tissue volumetry was performed in 42 obese subjects using abdominal CT scans.

Results The obese group included more women and subjects with type 2 diabetes mellitus, hypertension, and current smoking behavior. In obese subjects, HDL-C and triglycerides decreased with increasing BMI. Systolic and diastolic blood pressure, total cholesterol, LDL-C, and apoB were not related to BMI in the obese group, in contrast to the non-obese group. Inflammatory markers CRP, leukocyte count, and serum complement C3 increased with increasing BMI in the obese group, while these relations were less clear in the non-obese group. The subcutaneous adipose tissue surface was positively correlated to BMI, while no correlation was observed between BMI and visceral adipose tissue.

Conclusions Markers of inflammation are strongest related to BMI in obese subjects, most likely due to increased adipose tissue mass, while cardiovascular risk factors do not seem to deteriorate above a certain BMI level. Limited expansion capacity of visceral adipose tissue may explain these findings.

Keywords Morbid obesity · Cardiovascular risk · Inflammation · Atherosclerosis · Adipose tissue

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Introduction

Obesity has been associated to a higher prevalence of comorbidities, such as insulin resistance, type 2 diabetes mellitus (T2DM), hypertension (HT), and dyslipidemia [1, 2]. The risk for these obesity-related comorbidities is elevated with increasing body mass index (BMI), at least up to a BMI of 40 kg/m^2 [1]. Additionally, the excess body weight in obesity is known to increase all-cause mortality as well as cardiovascular mortality [2, 3], with the lowest mortality rates in subjects with a BMI between 20 and 25 kg/m^2 [2, 4]. An increase in BMI of 5 kg/m^2 can increase all-cause mortality with 30%, as well as mortality as a result of ischemic heart disease, stroke, and T2DM [2].

Although obesity may be an independent risk factor for cardiovascular disease (CVD), different studies attribute this effect of obesity mainly to differences in classic cardiovascular risk (CVR) factors between non-obese and obese subjects instead to obesity itself [5, 6]. Classic CVR factors are widely used to estimate the risk of CVD or mortality and include systolic and diastolic blood pressure, glycated hemoglobin (HbA1c), and dyslipidemia [7], and the values of these classic CVR factors increase with increasing BMI. More recently, interest has increased in inflammation as a risk factor for CVD [8], using C-reactive protein (CRP) and complement component C3 (C3) as markers of inflammation. Both CRP and C3 are associated with an increased risk of CVD [9–11] and increase with increasing BMI [12, 13].

In these studies, there has been an underrepresentation of morbidly obese subjects. Only few studies investigated the relationships of BMI and markers of dyslipidemia in morbid obesity, and these studies suggest an "obesity paradox" in which LDL-C levels are actually lower in subjects with the highest BMI, when compared to moderately obese subjects [14, 15], but these studies only focused on markers of dyslipidemia and not on other CVR factors.

The purpose of this study was to investigate the relationship of BMI with classic CVR factors and markers of inflammation in obese and non-obese subjects covering a wide range of BMI.

Materials and Methods

Design and Study Population

This was a cross-sectional study of obese and non-obese patients. This single-center case-control study included all morbidly obese patients who underwent preoperative screening for bariatric surgery from September 2009 to April 2011 in our bariatric clinic. All patients visiting the clinic for preoperative screening were asked to participate without any restrictions. Approximately 90% of all evaluated patients underwent surgery; the other 10% did not continue in the program due to several reasons. Inclusion criteria for bariatric surgery were as follows: age between 18 and 60 years old, BMI ≥ 40 kg/m^2 or BMI ≥ 35 kg/m^2 with obesity-associated disease. The reference population consisted of non-obese subjects with a BMI < 30 kg/m^2 participating in observational studies in our outpatient clinic [16, 17] from July 2009 to February 2013 and non-obese subjects referred to our clinic for cardiovascular risk management. These observational studies aimed to evaluate novel cardiovascular risk factors.

The cohort was divided in two groups according to BMI. The first group consisted of non-obese subjects, i.e., BMI < 30 kg/m^2, hereafter referred to as "non-obese subjects." The second group consisted of obese and morbidly obese subjects, i.e., BMI ≥ 30 kg/m^2, hereafter referred to as "obese subjects." Both subgroups were further divided into quintiles according to BMI.

Written informed consent was obtained from each individual and the study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki. For this type of study, no approval of the institution's ethics committee was required.

Baseline Characteristics

Baseline characteristics were collected according to a standard protocol in our clinic. Anthropometric measurements included weight, height, waist circumference, and blood pressure. BMI (kg/m^2) was calculated using both weight and height.

Laboratory Tests

Standard non-fasting screening laboratory tests were performed. Total cholesterol, HDL-C, and TG as well as glucose and inflammatory marker CRP were analyzed using the LX20 or DxC analyzers (Beckman Coulter, Miami, FL, USA). LDL-C values were calculated using the Friedewald formula. C3 and apolipoprotein B (apoB) were determined by rate nephelometry using IMAGE by commercially available kits (Beckman Coulter).

Adipose Tissue Depot Analyses Using Abdominal CT Scans

Volumetry measurements, using abdominal CT scans, were performed in order to analyze the relation between cardiovascular risk factors and the volume of visceral adipose tissue and subcutaneous adipose tissue. Within this cohort, 42 obese subjects underwent abdominal CT scans within a period of 2 weeks after bariatric surgery for clinical reasons. An abdominal CT scan is not part of the standard postoperative care in our department. The indication for these 42 abdominal CT scans was a (suspected) complication.

Two investigators independently measured volumes of sub-cutaneous and visceral adipose tissue in these scans. The CT scans were exported as "Digital Imaging and Communications in Medicine" (DICOM) data and analyzed using an open source image analysis software package, OsiriX® (version 7.0, 32-bit). The methods of CT volumetric analyses using OsiriX® have been described previously [18]. Adipose tissue was identified by Hounsfield units (HU) with a range between − 190 and − 30 HU [19] and measured on a single slice at level L4-L5, since the amount of visceral adipose tissue (VAT) at this level correlates best with total VAT volume [19]. The total amount of visceral adipose tissue was measured by selecting the abdominal cavity as "region of interest." The total amount of subcutaneous adipose tissue (SAT) was calculated by subtracting the amount of visceral adipose tissue from the total amount of adipose tissue. The mean adipose tissue surface of both investigators was used in the analysis. Interobserver reliability was analyzed by computing the two-way mixed absolute agreement single-measure intraclass correlation coefficient (ICC). The interobserver reliability was 0.961 for visceral adipose tissue ($p < 0.001$) and 0.980 for subcutaneous adipose tissue ($p < 0.001$).

Statistical Analysis

All analyses were performed using SPSS (PASW) 18.0 software (SPSS Inc., Chicago, IL, USA).

Continuous variables were presented as mean ± standard deviation (SD). Due to non-Gaussian distribution, both TG and CRP are described as median and minimum–maximum. Categorical data were described as an absolute number as well as a percentage of the total cohort.

Differences between obese and non-obese subjects were analyzed using independent T tests, chi-squared tests, and Kruskal-Wallis tests.

The relationship between BMI quintiles and metabolic and inflammatory parameters was analyzed using one-way ANOVA or the Kruskal-Wallis test in case of non-Gaussian distribution.

Patients on statins were excluded from the analyses on the relation between BMI and total cholesterol, HDL-C, LDL-C, TG, C3, CRP, and apoB. Subjects on antihypertensive drugs were excluded from analyses on the relation between BMI and systolic and diastolic blood pressure. Subjects on glucose-lowering drugs were excluded from the analysis on the relation between BMI and glucose. Pearson's correlation coefficients were calculated in order to analyze the relationship of BMI with the different adipose tissue surfaces. Results were evaluated at a 95% confidence interval at a significance threshold of $p < 0.05$ (two-sided).

Results

The obese group consisted of 576 subjects (418 women and 158 men), with a mean age of 44.2 (± 13.0) years and a mean

BMI of 43.8 (± 7.6) kg/m². The non-obese group consisted of 377 subjects (173 women and 204 men). The mean age was 58.7 (± 13.0) years and the mean BMI was 25.0 (± 2.81) kg/m². Additional baseline characteristics of both groups are displayed in Table 1. The obese group included significantly more women than the non-obese group and was significantly younger. T2DM, HT, and current smoking behavior were more prevalent in the obese group. The obese group had a significantly higher BMI and waist circumference, when compared to the non-obese subjects. Focusing on classic cardiovascular risk factors, the mean systolic and diastolic blood pressure, LDL-C, and glucose levels were significantly higher in the obese subjects then those in non-obese subjects, while HDL-C was lower in the obese group. ApoB, C3, and CRP, which are thought to be related to cardiovascular risk, were also elevated in the obese subjects. Only total cholesterol levels and triglyceride levels were not different between the groups (Table 1).

In both the obese and non-obese group, no clear relation was observed between the level of BMI and the level of systolic blood pressure. In contrast, diastolic blood pressure increased with increasing BMI in the non-obese group, but no relation was observed between the level of diastolic blood pressure and BMI in the obese group. A similar trend was observed in the level of LDL-C and apoB in relation to BMI in both groups.

HDL-C decreased with increasing BMI in both groups, although the decrease in the obese group stabilizes from the second quintile and up. The significant decrease can be solely explained by a relatively high HDL-C level in the first BMI quintile of the obese group. In contrast to all other classic cardiovascular risk factors, the triglyceride level showed a gradual increase with increasing BMI in the non-obese group, but a gradual decrease in the obese group (Tables 2 and 3).

Inflammatory markers, which are also related to cardiovascular risk, followed a different pattern with BMI in both groups. Both CRP and leukocyte count were not related to BMI in the non-obese group. However, both parameters showed a clear relationship with BMI in the obese group. Complement C3 was the only parameter with a positive relationship with BMI in both the obese and non-obese groups (Tables 2 and 3).

Adipose Tissue in Subcutaneous and Visceral Depots by CT Scans

Forty-two abdominal CT scans of obese patients were performed in the perioperative period for bariatric surgery. No significant differences in baseline characteristics were seen between obese subjects who did or did not undergo an abdominal CT scan. A positive correlation of BMI with subcutaneous adipose tissue surface was found ($r = 0.633$, $p < 0.001$), while no significant correlation of BMI with visceral adipose tissue

Table 1 Baseline characteristics of both obese and non-obese subjects in absolute numbers or mean value, with its percentage or SD, respectively, presented within brackets

	Non-obese ($n = 377$)	Obese ($n = 576$)	p value
Sex (%)°, female	173 (45.9%)	418 (72.6%)	$p < 0.001$
Age* (years)	58.7 (± 13.0)	44.2 (± 13.0)	$p < 0.001$
BMI* (kg/m^2)	25.0 (± 2.81)	43.8 (± 7.58)	$p < 0.001$
Waist circumference* (cm)	93.5 (± 10.6)	128.6 (± 16.3)	$p < 0.001$
Medical history			
Type 2 diabetes°	41 (10.9%)	132 (22.9%)	$p < 0.001$
Hypertension°	107 (28.4%)	199 (34.5%)	$p = 0.019$
Dyslipidemia°	73 (19.4%)	96 (16.7%)	$p = 0.436$
Current smoking			$p = 0.031$
Yes	70 (18.6%)	286 (24.3%)	
No	306 (81.2%)	284 (74.7%)	
Systolic blood pressure* (mmHg)	125 (± 14.3)	138 (± 16.0)	$p < 0.001$
Diastolic blood pressure* (mmHg)	76 (± 9.6)	84 (± 10.2)	$p < 0.001$
Total cholesterol* (mmol/l)	5.26 (± 1.28)	5.23 (± 0.99)	$p = 0.745$
HDL-cholesterol* (mmol/l)	1.49 (± 0.45)	1.20 (± 0.30)	$p < 0.001$
LDL-cholesterol* (mmol/l)	3.18 (± 1.11)	3.41 (± 0.96)	$p = 0.008$
Triglycerides† (mmol/l)	1.16 (0.3–9.7)	1.18 (0.3–9.5)	$p = 0.296$
ApoB* (g/l)	0.97 (± 0.305)	1.08 (± 0.264)	$p < 0.001$
CRP† (nmol/l)	19 (9.5–1542)	67 (9.5–486)	$p < 0.001$
Complement C3* (mg/l)	1.07 (± 0.226)	1.65 (± 0.275)	$p < 0.001$
Leukocytes* (10^9/l)	6.6 (± 1.78)	8.5 (± 2.32)	$p < 0.001$
Glucose* (mmol/l)	5.7 (± 1.59)	6.5 (± 1.69)	$p < 0.001$

°Group differences were tested using χ^2

*Group differences were tested using independent T tests

† Described as median (minimum–maximum) and analyzed using the Kruskal-Wallis test

surface was observed ($r = -0.068$, $p = 0.670$) (Fig. 1). There were no significant correlations between the surface areas of visceral and subcutaneous adipose tissue on the one hand and the classic CVR factors or inflammatory markers on the other.

Discussion

Although diastolic and systolic blood pressure and the levels of LDL-C, glucose, and apoB were significantly increased in our obese subjects, the level of BMI in this group did not seem to influence the level of CVR factors. Therefore, derangement of CVR factors overall appears to reach a plateau at a certain level of obesity. A clear explanation for these observations is lacking.

One explanation for this finding may be the different metabolic effects of visceral and subcutaneous adipose tissue. Central adiposity is strongly associated with metabolic disturbances, such as insulin resistance, dyslipidemia, and systemic inflammation, which play essential roles in the pathogenesis of CVD [20, 21]. Furthermore, it is also associated with both cardiovascular mortality, cancer mortality, and overall mortality [20]. More specifically, fat distribution may play an

important role in the risk of metabolic disease and CVD [22], in which visceral adipose tissue is most strongly related to measures of metabolic disease [21]. The present study suggests that visceral adipose tissue appears to have limited potential for expansion. Therefore, it can be hypothesized that after saturation of the visceral adipose tissue depot, further increases in obesity, e.g., BMI, result in fat storage in other depots, such as subcutaneous adipose tissue. As a result, the detrimental effects of visceral adipose tissue will not increase with increasing BMI in morbid and superobese subjects, as suggested by the present data. Expansion of subcutaneous adipose tissue at the expense of visceral adipose tissue may protect obese subjects from further deterioration of their CVR factors. The "adipose tissue expandability model" states that adipose tissue in general has a maximum potential for expansion in a given individual [23]. Once this degree of maximal expansion is reached, the adipose tissue is no longer able to safely store excess energy and the lipid flux to non-adipose organs will increase, resulting in ectopic fat accumulation. Storage of lipids in ectopic sites, such as hepatocytes or beta cells, can eventually result in metabolic disturbances as seen in obese patients [24, 25]. However, none of these studies evaluated the expansion capacity of visceral and subcutaneous

Table 2 Mean value (±SD) of cardiovascular risk factors and inflammatory markers in the different quintiles based on BMI of the obese group

	First quintile, BMI 33.8 (± 2.39)	Second quintile, BMI 39.9 (± 1.04)	Third quintile, BMI 43.0 (± 0.93)	Fourth quintile, BMI 47.1 (± 1.49)	Fifth quintile, BMI 54.9 (± 5.26)	p value*
Age (years)	56.4 (± 9.8), $n = 115$	43.5 (± 11.3), $n = 115$	40.6 (± 10.3), $n = 116$	38.8 (± 11.5), $n = 115$	41.6 (± 11.6), $n = 115$	$p < 0.001$
Sex (female)	59 (51.3%)	84 (73.0%)	92 (79.3%)	94 (81.7%)	89 (77.4%)	$p < 0.001$
Systolic blood pressure (mmHg)	132 (± 14.3), $n = 33$	137 (± 14.9), $n = 62$	139 (± 16.3), $n = 73$	139 (± 15.4), $n = 76$	142 (± 18.2), $n = 54$	$p = 0.096$
Diastolic blood pressure (mmHg)	82 (± 9.5), $n = 33$	84 (± 9.6), $n = 62$	84 (± 9.3), $n = 73$	85 (± 11.3), $n = 76$	85 (± 10.8), $n = 54$	$p = 0.581$
Total cholesterol (mmol/l)	5.4 (± 1.1), $n = 58$	5.4 (± 1.1), $n = 81$	5.1 (± 1.0), $n = 98$	5.2 (± 0.9), $n = 92$	5.1 (± 1.0), $n = 94$	$p = 0.063$
HDL-cholesterol (mmol/l)	1.33 (± 0.37), $n = 58$	1.22 (± 0.27), $n = 81$	1.17 (± 0.27), $n = 98$	1.15 (± 0.29), $n = 92$	1.19 (± 0.30), $n = 94$	$p = 0.005$
LDL-cholesterol (mmol/l)	3.4 (± 1.0), $n = 56$	3.6 (± 1.0), $n = 79$	3.3 (± 0.9), $n = 95$	3.5 (± 1.0), $n = 91$	3.3 (± 0.9), $n = 92$	$p = 0.422$
Triglycerides[†] (mmol/l)	1.52 (0.5–9.5), $n = 58$	1.18 (0.3–5.3), $n = 81$	1.14 (0.3–4.9), $n = 98$	1.14 (0.3–8.1), $n = 92$	1.10 (0.4–4.4), $n = 93$	$p = 0.009$
ApoB (g/l)	1.06 (± 0.276), $n = 57$	1.11 (± 0.287), $n = 81$	1.07 (± 0.267), $n = 98$	1.09 (± 0.257), $n = 92$	1.04 (± 0.238), $n = 94$	$p = 0.420$
CRP[†] (nmol/l)	33 (9.5–429), $n = 48$	57 (9.5–238), $n = 64$	76 (9.5–486), $n = 83$	86 (9.5–286), $n = 64$	100 (9.5–476), $n = 70$	$p < 0.001$
Complement C3 (mg/l)	1.43 (± 0.259), $n = 57$	1.61 (± 0.244), $n = 81$	1.66 (± 0.229), $n = 98$	1.67 (± 0.257), $n = 92$	1.81 (± 0.276), $n = 93$	$p < 0.001$
Leukocytes (10^9/l)	7.5 (± 2.45), $n = 111$	8.5 (± 2.14), $n = 111$	8.7 (± 2.27), $n = 115$	8.7 (± 2.04), $n = 112$	9.3 (± 2.34), $n = 114$	$p < 0.001$
Glucose (mmol/l)	6.4 (± 1.58), $n = 76$	6.8 (± 2.15), $n = 81$	6.6 (± 2.14), $n = 98$	6.2 (± 0.94), $n = 86$	6.3 (± 1.21), $n = 83$	$p = 0.165$

*Group differences were tested using one way ANOVA

[†] Described as mean (minimum–maximum) and analyzed using the Kruskal-Wallis test

adipose tissue in the course of increasing obesity in humans. It should be noted that a limitation of the present study is the small number of abdominal CT scans in the obese group. Furthermore, due to the lack of abdominal CT scans in the non-obese group, our data concerning the different adipose tissue depots only apply to obese subjects. Future studies

Table 3 Mean value (±SD) of cardiovascular risk factors and inflammatory markers in the different quintiles based on BMI of the non-obese group

	First quintile, BMI 20.8 (± 1.45)	Second quintile, BMI 23.6 (± 0.67)	Third quintile, BMI 25.3 (± 0.34)	Fourth quintile, BMI 26.6 (± 0.54)	Fifth quintile, BMI 28.8 (± 0.69)	p value*
Age (years)	53.8 (± 15.1), $n = 75$	60.4 (± 12.7), $n = 77$	59.6 (± 12.1), $n = 74$	58.0 (± 13.7), $n = 76$	61.5 (± 9.8), $n = 75$	$p = 0.003$
Sex (female)	47 (62.7%)	43 (55.8%)	26 (35.1%)	36 (47.4%)	21 (28.0%)	$p < 0.001$
Systolic blood pressure (mmHg)	124 (± 13.9), $n = 52$	125 (± 14.4), $n = 40$	126 (± 15.4), $n = 24$	122 (± 13.6), $n = 33$	132 (± 13.8), $n = 23$	$p = 0.118$
Diastolic blood pressure (mmHg)	73 (± 8.5), $n = 52$	74 (± 10.1), $n = 40$	81 (± 9.9), $n = 24$	76 (± 9.0), $n = 33$	80 (± 8.8), $n = 23$	$p = 0.002$
Total cholesterol (mmol/l)	4.9 (± 1.4), $n = 58$	5.2 (± 1.4), $n = 46$	5.6 (± 1.1), $n = 34$	5.5 (± 1.0), $n = 46$	5.4 (± 1.3), $n = 24$	$p = 0.144$
HDL cholesterol (mmol/l)	1.62 (± 0.46), $n = 58$	1.53 (± 0.45), $n = 46$	1.50 (± 0.38), $n = 34$	1.36 (± 0.44), $n = 46$	1.32 (± 0.46), $n = 24$	$p = 0.013$
LDL cholesterol (mmol/l)	2.8 (± 1.2), $n = 58$	3.1 (± 1.2), $n = 46$	3.5 (± 1.0), $n = 34$	3.4 (± 0.9), $n = 44$	3.4 (± 1.2), $n = 23$	$p = 0.034$
Triglycerides[†] (mmol/l)	0.97 (0.3–3.4), $n = 58$	1.05 (0.4–2.8), $n = 46$	1.13 (0.6–3.2), $n = 34$	1.37 (0.4–9.7), $n = 46$	1.28 (0.6–4.8), $n = 24$	$p = 0.001$
ApoB (g/l)	0.86 (± 0.285), $n = 58$	0.95 (± 0.354), $n = 46$	1.03 (± 0.246), $n = 34$	1.06 (± 0.260), $n = 46$	1.05 (± 0.339), $n = 24$	$p = 0.005$
CRP[†] (mg/l)	9.5 (9.5–295), $n = 56$	14 (9.5–619), $n = 44$	19 (9.5–105), $n = 32$	19 (9.5–200), $n = 44$	29 (9.5–1543), $n = 24$	$p = 0.069$
Complement C3 (mg/l)	0.96 (± 0.189), $n = 58$	1.07 (± 0.229), $n = 46$	1.11 (± 0.228), $n = 34$	1.14 (± 0.225), $n = 46$	1.18 (± 0.203), $n = 24$	$p < 0.001$
Leukocytes (10^9/l)	6.5 (± 1.89), $n = 75$	6.6 (± 1.99), $n = 77$	6.4 (± 1.81), $n = 74$	6.5 (± 1.37), $n = 76$	7.1 (± 1.72), $n = 75$	$p = 0.142$
Glucose (mmol/l)	5.5 (± 1.53), $n = 75$	5.7 (± 1.67), $n = 72$	5.8 (± 1.83), $n = 68$	5.5 (± 1.14), $n = 71$	6.2 (± 1.64), $n = 64$	$p = 0.082$

*Group differences were tested using one way ANOVA

[†] Described as mean (minimum–maximum) and analyzed using the Kruskal-Wallis test
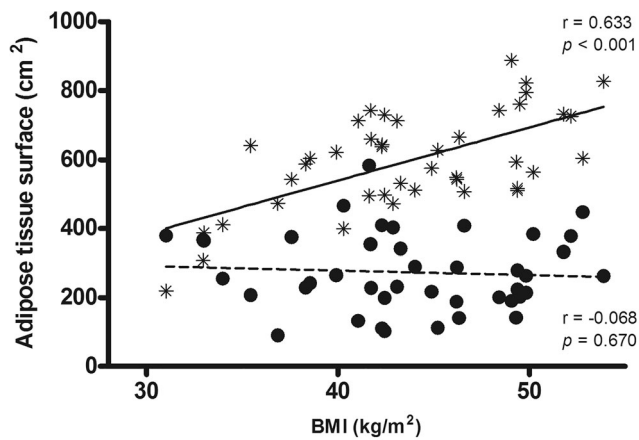
Continuous line w ith asterisk represents subcutaneous adipose tissue. The dotted line w ith circles represents visceral adipose tissue.

Fig. 1 Correlation of BMI and adipose tissue surfaces in obese subjects ($n = 42$)

investigating this issue should include measurements of adipose tissue depots.

In contrast to the previously mentioned CVR factors, inflammatory markers showed a clear relationship with the level of obesity in the obese group, while CRP and leukocyte count did not show an association with BMI in the non-obese group. Adipose tissue is known to secrete several adipokines that in part may cause increases in CRP as suggested by the relationship found in obese subjects between BMI and CRP. We did not use a high-sensitive CRP assay, which may in part explain the lack of association in the non-obese subjects. However, the inflammatory marker C3 did increase with increasing BMI in both the non-obese and obese groups, and C3 levels have been shown to be associated with CRP levels [26]. Although systemic inflammation is positively related to BMI, classical CVR factors seem to reach a maximal level at BMI 35–40 kg/m^2. Increased cardiovascular risk in subjects with BMI > 40 kg/m^2 may depend more on systemic inflammation and less so on classic CVR factors.

One unexpected finding of this study was the paradoxical decrease in TG with increasing BMI in our obese subjects, causing a peak level of TG within the group of subjects with a BMI between 26 and 35 kg/m^2. A limitation in our measurement of TG is that fasting venipuncture was not a requirement within our cohort; we were unable to distinguish between fasting and non-fasting subjects. However, the latest guidelines on lipid measurements question the need for fasting measurements since normal food intake does not largely affect lipid levels and the intra-individual variability in TG remains comparable throughout the day [27, 28]. Additionally, it is unlikely that non-fasting TG levels were mainly measured in subjects with a BMI between 26 and 35 kg/m^2. Therefore, we assume that the combination of fasting and non-fasting TG levels cannot explain the paradoxical trend in TG levels in the obese group. Porter et al. [29] previously noticed an

increase of TG levels with increasing visceral fat volume. However, in the group with the highest visceral fat volumes, TG levels decreased with increasing subcutaneous fat volumes [29], which is in line with our findings. They suggested that subcutaneous adipose tissue may have beneficial effects on triglyceride metabolism in those subjects with large visceral adipose tissue volumes. Unfortunately, the molecular mechanism behind these findings has not been determined yet.

Even though excess body weight is known to increase all-cause mortality and cardiovascular mortality, previous studies only included subjects with a BMI up to 35 kg/m^2 [5, 20, 22]. However, 1.5 to 6% of all adults in developed countries are known to be morbidly obese [30], with a BMI of > 40 kg/m^2, and this prevalence is still rising. Much interest exists on the treatment of these morbidly obese subjects, in order to achieve significant weight loss and resolution of obesity-related comorbidity and thereby prevention of preterm mortality. The Prospective Studies Collaboration has demonstrated that overall mortality, as well as cardiovascular mortality, increases with increasing BMI, at least up to a BMI of 50 kg/m^2 [2]. Furthermore, overweight and obesity are associated with an early onset of CVD, not only resulting in higher mortality, but also in a greater portion of lives lived with CVD morbidity [31]. These findings should urge clinicians to intensify cardiovascular risk management in obese subjects. The current cardiovascular risk management should not be simply assumed to be suitable for morbidly obese patients. Previous studies reveal that hypertension in obese patients is of a different phenotype than hypertension in the lean population. In addition, not all antihypertensive drugs appear to be equally effective in obesity-related hypertension as in hypertension in lean patients [32, 33]. Furthermore, current guidelines for the treatment of dyslipidemia may not be suitable in obesity. Obese subjects are thought to require more intensive treatment for dyslipidemia with higher doses of lipid-lowering drugs [34]. Nevertheless, our data suggest that classic CVR factors do not further deteriorate with increasing BMI, from a BMI of approximately 35 kg/m^2 and higher. This suggests that the increased cardiovascular mortality in obesity is not caused by deterioration of classic cardiovascular risk factors, but that obesity is an independent cardiovascular risk factor itself. The increased cardiovascular mortality in obese patients [2, 35] may be influenced by other factors, such as systemic inflammation or non-atherosclerotic heart disease. Future studies should distinguish between different cardiovascular mortality causes in morbidly obese subjects, such as atherosclerotic heart disease, hypertensive heart disease, cardiomyopathies, or heart failure [36].

The distinct difference in baseline characteristics between the non-obese and obese groups in this study is a major limitation, even though the results were adjusted for comorbidity. When interpreting our results, we should keep in mind that patients on antihypertensive or lipid-lowering drugs were excluded from the analysis when the drug would interfere with the

cardiovascular risk factor under investigation. Therefore, the relationships may not be applicable in subjects who already receive treatment for cardiovascular risk reduction. These excluded subjects could elevate the level of the specific risk factor if they were added to the analysis after cessation of their therapy. However, we do not have these data and felt that the use of antihypertensive and lipid-lowering drugs would disturb the natural relationship between BMI and the risk factors. Therefore, these confounding factors were excluded with the realization that the data may be biased. Regardless of this limitation, the results of this study provide new insights in cardiovascular risk in a population of high interest, since obesity and morbid obesity are reaching epidemic proportions [37] with substantial economic burden, not only in terms of medical costs, but also in terms of non-medical costs (e.g., absenteeism and personal costs) [38]. Our future perspective is to analyze cardiovascular risk factors in relation to BMI in a larger population in which we are able to match on age and gender and correct for confounding factors. The level of obesity should become a part of the currently available cardiovascular risk calculators.

In conclusion, obesity is related to an increased risk on metabolic and CVD and mortality, but this increased risk may not be solely explained by deterioration of classic CVR factors. The lack of correlation of CVR factors and BMI in obese subjects may be explained by the expansion of subcutaneous adipose tissue with increasing BMI after saturation of the visceral adipose tissue compartment. In order to reduce the risk of CVD and mortality in obese subjects, treatment may need to focus on reduction of systemic inflammation and on non-atherosclerotic heart diseases.

Author Contributions SvM has a substantial contribution in the conception and design of the study, participated in analysis and interpretation of data, and drafted the manuscript. GV participated in the CT scan analysis and has a substantial contribution to drafting and revising the manuscript for intellectual content. AvH and BK participated in data collection and revising the manuscript for intellectual content. GJvdG participated in study conception and in revision of the manuscript. EB participated in the statistical analysis and in revision of the manuscript. GJB, GM, and LB contributed to revising the manuscript for intellectual content. MCC has a substantial contribution to the conception and design of the study and revising the manuscript for intellectual content.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Informed Consent Informed consent was obtained from all individual participants included in the study.

Human and Animal Right All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Ethical Approval For this type of study, formal consent is not required.

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