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The Role of Visceral and Subcutaneous Adipose Tissue Measurements and their Ratio by Magnetic Resonance Imaging in Subjects with Prediabetes, Diabetes and Healthy Controls from a General Population without Cardiovascular Disease

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Abstract:	Objectives: To study the relationship of area- and volumetric-based visceral and subcutaneous adipose tissue (VAT and SAT) by magnetic resonance imaging (MRI) and their ratio in subjects with impaired glucose metabolism from the general population. Methods: Subjects from a population-based cohort with established prediabetes, diabetes and healthy controls without prior cardiovascular diseases underwent 3 Tesla MRI. VAT and SAT were as total volume and area on a single slice, and their ratio (VAT/SAT) was calculated. Clinical covariates and cardiovascular risk factors were assessed in standardized fashion. Univariate and adjusted analyses were conducted. Results: Among 384 subjects (age: 56.2 ± 9.2 years, 58.1% male) with complete MRI data available, volumetric and single-slice VAT, SAT and VAT/SAT ratio were strongly

	correlated (all > r=0.89). Similarly, VAT/SATvolume ratio was strongly correlated with VATvolume but not with SAT (r=0.72 and r=-0.21, respectively). Significant higher levels of VAT, SAT and VAT/SAT ratio were found in subjects with impaired glucose metabolism (all p≤0.01). After adjustment for potential cardiovascular confounders, VATvolume and VAT/SATvolume ratio remained significantly higher in subjects with impaired glucose metabolism (all <0.02), whereas the association for SATvolume attenuated. Additionally, there was a decreasing effect of glycemic status on VAT/SATvolume ratio with increasing body mass index and waist circumference. Conclusions: VATvolume and VAT/SATvolume ratio are associated with impaired glucose metabolism, independent of cardiovascular risk factors or MRI-based quantification technique, with a decreasing effect of VAT/SATvolume ratio in obese subjects. Advances in knowledge: Quantification of VATvolume and VAT/SATvolume ratio by MRI represents a reproducable biomarker for cardiometabolic risk in subjects with impaired glucose metabolism, however, there seems to be an attenuated association of VAT/SATvolume ratio with glycemic state in obese subjects.
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The Role of Visceral and Subcutaneous Adipose Tissue Measurements and their Ratio by Magnetic Resonance Imaging in Subjects with Prediabetes, Diabetes and Healthy Controls from a General Population without Cardiovascular Disease

Running title: The role of adipose tissue ratio by MRI in diabetes

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Dear Prof. Prise and Dr. Jackson,

Thank you very much for the opportunity to submit our research manuscript to your upcoming special feature on the role of imaging in obesity. Please find attached our original manuscript entitled "The Role of Visceral and Subcutaneous Adipose Tissue Measurements and their Ratio by Magnetic Resonance Imaging in Subjects with Prediabetes, Diabetes and Healthy Controls from a General Population without Cardiovascular Disease".

As to our previous conversation, in this prospective, population-based cohort study, we studied the relationship of area- and volumetric-based visceral and subcutaneous adipose tissue (VAT and SAT) by magnetic resonance imaging (MRI) and their ratio in subjects with impaired glucose metabolism from the general population. We found a very strong correlation between MRI-based volumetric and single-sliced measurements of VAT and SAT and the VAT/SAT ratio. Furthermore, increased VAT and VAT/SAT ratios were associated with prediabetes and diabetes, independent of cardiometabolic confounders. Interestingly, our results indicate, that there is an attenuated association attenuated association of VAT/SAT_{volume} ratio with glycemic state in obese subjects with high BMI or waist cirumference, possibly dominated by the variation of VAT_{volume} in obese subjects.

This manuscript is not under consideration elsewhere, none of the paper's contents have been previously published in this form, all authors have read and approved the manuscript, we have nothing to disclose, and there is no conflict of interest.

Thank you for considering our manuscript. We would be happy to make any further changes necessary to meet the requirements of your journal.

Sincerely,

Corinna Storz, Sophia Heber and Fabian Bamberg
On behalf of the authors

Manuscript

ABSTRACT

Objectives: To study the relationship of area- and volumetric-based visceral and subcutaneous adipose tissue (VAT and SAT) by magnetic resonance imaging (MRI) and their ratio in subjects with impaired glucose metabolism from the general population.

Methods: Subjects from a population-based cohort with established prediabetes, diabetes and healthy controls without prior cardiovascular diseases underwent 3 Tesla MRI. VAT and SAT were as total volume and area on a single slice, and their ratio (VAT/SAT) was calculated. Clinical covariates and cardiovascular risk factors were assessed in standardized fashion. Univariate and adjusted analyses were conducted.

Results: Among 384 subjects (age: 56.2 ± 9.2 years, 58.1% male) with complete MRI data available, volumetric and single-slice VAT, SAT and VAT/SAT ratio were strongly correlated (all > r=0.89). Similarly, VAT/SAT_{volume} ratio was strongly correlated with VAT_{volume} but not with SAT (r=0.72 and r=-0.21, respectively). Significant higher levels of VAT, SAT and VAT/SAT ratio were found in subjects with impaired glucose metabolism (all p≤0.01). After adjustment for potential cardiovascular confounders, VAT_{volume} and VAT/SAT_{volume} ratio remained significantly higher in subjects with impaired glucose metabolism (all <0.02), whereas the association for SAT_{volume} attenuated. Additionally, there was a decreasing effect of glycemic status on VAT/SAT_{volume} ratio with increasing body mass index and waist circumference.

Conclusions: VAT_{volume} and VAT/SAT_{volume} ratio are associated with impaired glucose metabolism, independent of cardiovascular risk factors or MRI-based quantification technique, with a decreasing effect of VAT/SAT_{volume} ratio in obese subjects.

Advances in knowledge: Quantification of VAT_{volume} and VAT/SAT_{volume} ratio by MRI represents a reproducable biomarker for cardiometabolic risk in subjects with impaired

glucose metabolism, however, there seems to be an attenuated association of VAT/SAT_{volume} ratio with glycemic state in obese subjects.

INTRODUCTION

Diabetes is a common widespread disease with a steadily increasing prevalence worldwide. Age-standardized global prevalence of diabetes has almost doubled since 1980, rising from 4.7% to 8.5%, identifying diabetes as one of the leading growing health challenges (1). Patients with diabetes were previously shown to have a 2- to 3- fold higher risk for the development of cardiovascular diseases (2). Furthermore, obesity, defined by a body mass index (BMI) of at least 30 kg/m^2, is a strong predictive factor in the development of type 2 diabetes, and obesity, in turn, represents a major risk factor for cardiovascular diseases such as coronary heart disease (3, 4).

There is early evidence, that BMI seems to be a valid indicator for the overall identification of obesity, however, previous research has identified individual distribution and functional differences of several fat compartments (5-7). Furthermore, early studies have determined an association of different fat compartments with different metabolic risk, especially insulin resistance (5-9). As a ratio of the body mass divided by the square of the body height, the BMI does not factor in the distribution of muscle and adipose tissue in individuals. Moreover, ethnical differences make BMI a rather inconsistent tool for estimating body composition (10).

Specifically, visceral adipose tissue (VAT) seems to be more strongly associated with metabolic risk and is often considered to be a unique pathogenic adipose tissue depot, associated with adverse outcome and higher metabolic risk (5, 11, 12). Besides dyslipidemia, for instance, it is well established that impaired glucose metabolism is associated with VAT (13). Furthermore, other ectopic fat depots such as epicardial fat are associated with VAT (14) and it has become clear that adipocytes in VAT display a broader spectrum of inflammatory mediators than other fat depots (15). Notably, there is also early evidence that VAT is associated with specific genetic predispositions in women (16).

The contributing role of subcutaneous adipose tissue (SAT) in the development of metabolic syndrome is still controversial. Moreover, several studies indicated that SAT may have beneficial effects on metabolism, emphasizing the intrinsic difference in adipose depots independent of the anatomic location (9, 17, 18). In contrary, excess SAT has also been suggested to contribute to metabolic syndrome (19). Molecular studies previously showed that VAT is associated with a higher production of inflammatory cytokines leading to an increased metabolic activity, as it secrets more humoral mediators such as adiponectin and leptin, and therefore carries a greater predicition for mortality than SAT (20). However, the complexity of anatomic and functional fat depots such as VAT and SAT remains poorly understood.

The various fat compartments can be quantified non-invasively by magnetic resonance imaging (MRI) (21). Compared to other imaging modalities, such as ultrasound (US), computed tomography (CT), or dual X-ray absorptiometry (DXA) (22-26), MRI may represent a value tool in the prevention setting, due to its non-ionizing nature (27). However, there are a number of different parameters available, including volumetric and area-based estimates of fat depots at different transverse levels of the torso (28, 29). Earlier research has focused on the ratio between VAT and SAT (VAT/SAT ratio) as a metric of individual body fat, which has been shown to represent a predictor of cardiac events and adverse outcome, independent of the absolute fat volume (30, 31).

Therefore, the aim of this study was to systematically study the association between the different parameters of fat depots obtainable by MRI and impaired glucose metabolism in subjects from the general population without cardiovascular disease. Our hypothesis was that there are parameters that are more strongly associated with diabetes status than others.

METHODS

Study Population

The study was designed as a case control study nested in a prospective cohort from the "Cooperative Health Research in the Region of Augsburg" (KORA) between June 2013 and September 2014 and previously described elsewhere (32, 33). An oral glucose tolerance test was administered to all participants who had not been diagnosed for type-2 diabetes, and established definitions of diabetes and prediabetes were applied (34, 35). Other established risk factors were collected in standardized fashion as part of the KORA study design, as previously described (32, 33).

Subjects were eligible if they met the following inclusion criteria: a) willingness to undergo whole-body MRI and b) qualification in either the prediabetes, diabetes, or control group, according to the definition of the World Health Organisation (34). Subjects, who met the following criteria, were excluded: a) age above 72 years, b) subjects with prior cardiovascular diseases, c) contraindications against standard MRI exam such as cardiac pacemaker, surgical clip material, pregnancy or breastfeeding subjects, or subjects with claustrophobia, known allergy against gadolinium compounds, or an impaired renal function with a serum creatinine ≥1.3 mg/dL.

Systolic and diastolic blood pressure measurements were obtained three times at the right arm of seated subjects after a 5-min resting period; the mean of the second and third measurements was used for analyses. Hypertension was defined as increased systolic blood pressure ≥140mmHg, increased diastolic blood pressure ≥90mmHg or intake of antihypertensive medication under awareness of having hypertension. Subjects who reported current regular or sporadic cigarette smoking were defined as smokers, those who reported only previous regular or sporadic cigarette smoking were defined as ex-smokers; all others were defined as never smokers.

The study was approved by the institutional review board of the medical faculty of the Ludwig-Maximilian University Munich and all participants provided written informed consent prior to the commencement of the study.

Magnetic Resonance Imaging for Assessment of Body Adipose Tissue Compartments

The body adipose protocol was embedded in a comprehensive, whole-body exam using a 3 Tesla Magnetom Skyra (Siemens AG, Healthcare Sector, Erlangen, Germany) as detailed described elsewhere (32). This protocol comprised a three-dimensional in/opposed-phase VIBE-Dixon sequence using the following parameters: Slice Thickness 1.7 mm, spatial resolution: 1.7 x 1.7 mm^2, FOV: 488 x 717 mm using a 256 x 256 mm matrix ,TR: 4.06 ms TE: 1.26; 2.49 ms, with a 9 degrees flip angle.

Based on the volume-interpolated three-dimensional in/opposed-phase VIBE-Dixon sequence a fat selective tomogram was reconstructed (slice thickness 5mm at 5mm increment). For semi-automatically quantification of the adipose tissue compartments, an in-house algorithm based on Matlab R2013a was used (21). The volumetric VAT_{volume} was measured from the femoral head to the cardiac apex, the volumetric SAT_{volume} was calculated from the femoral head to the diaphragm, indicated in liter (l). The volumetric total adipose tissue (TAT_{volume}) is defined as the summary of VAT_{volume} and SAT_{volume}, calculated from the femoral head to the diaphragm and cardiac apex, respectively, indicated in liter (l). In addition, both visceral and subcutaneous adipose tissue compartments were measured at a single slice at the level of the umbilicus based on a VIBE-Dixon sequence (VAT_{area} and SAT_{area}, respectively), indicated in square centimeter (cm^2). All segmentations were manually adjusted if necessary. An example of the VAT and SAT compartments as total volume and area on a single slice, in a control and a subject with prediabetes is depicted in Figure 1.

All analyses were performed in a blinded fashion by independent readers unaware of the glycemic status and clinical covariates.

Statistical Analysis

Demographic characteristics, risk factors and adipose tissue parameters of participants are presented as arithmetic means and standard deviations for continuous variables and counts and percentages for categorical variables. A two-sample t-test with pooled variance was used to analyze differences in mean adipose tissue variables. The correlation between the respective adipose tissue parameters with the corresponding confidence interval was calculated by Pearson's correlation coefficient and correlation was interpreted as very weak (r=0-0.19), weak (r=0.20-0.39), moderate (r=0.40-0.59), strong (r=0.60-0.79) and very strong (r=0.80-1.00) (36).

The association of body adipose tissue on glycemic status was evaluated by an ordered logistic regression model adjusted for age and sex. The association of glycemic status to body adipose tissue was assessed by linear regression models adjusted for age, sex, smoking, body mass index, hypertension, high density lipoprotein (HDL), low density lipoprotein (LDL) and triglycerides. Interactions of glycemic status and BMI/Waist circumference were evaluated by calculating marginal effects based on linear regression models including multiplicative interaction terms. P-values < 0.05 were considered to denote statistical significance. All calculations were performed with R v3.4.1.

RESULTS

Among 400 subjects enrolled, a total of 384 subjects with complete MR datasets were included in the final analysis (96.0%). Of them, 235 were healthy controls, 97 were classified as prediabetes and 52 with diabetes (61.2%, 25.3% and 13.5%, respectively). The mean age was 56.2 ± 9.2 years and 58.1% of the subjects were male (Table 1).

Correlation Between Different MR-Parameters of Fat Depots

Volumetric and single-sliced VAT and SAT were strongly correlated independent of measurement technique (Figure 2, r=0.92 and r=0.95, respectively). SAT and VAT were moderately correlated, independent of measurement technique (r=0.43 for and r=0.39 for volumetric and single-sliced measurements, respectively). However, the correlations between volumetric and single-sliced VAT/SAT ratios were strong (r=0.89). Thus, all subsequent analysis was carried out using the volumetric measurement. Comparing VAT/SAT_{volume} ratios with the respective VAT_{volume} and SAT_{volume}, we found a strong correlation between the VAT/SAT_{volume} ratio and the respective VAT_{volume} (Figure 3, r=0.72). VAT/SAT_{volume} ratio and SAT_{volume} or TAT_{volume} were weakly (r=0.21 for VAT/SAT_{volume} and TAT_{volume}) or not correlated (r=-0.21 for VAT/SAT_{volume} and SAT_{volume}).

Association of MR Parameters with Glycemic Status

There were significant differences in the several fat depots between the subgroups (Table 2). TAT_{volume}, SAT_{volume} and VAT_{volume} were significantly higher in subjects with prediabetes and diabetes as compared to healthy controls (all $p \leq 0.001$). Also, the VAT/SAT_{volume} ratio was significantly higher in subjects with prediabetes and diabetes. The association between VAT_{volume} and glycemic status (odds ratio (OR): 3.1) was stronger than for

SAT_{volume} (OR: 2.1), VAT/SAT_{volume} ratio (OR: 2.0), BMI (OR:2.1) or waist circumference (OR:2.6).

After adjustment for potential confounders, including age, sex and hypertension, prediabetes and diabetes remained significantly associated with TAT_{volume}, SAT_{volume}, VAT_{volume} and VAT/SAT_{volume} ratio (all $p \leq 0.006$, Table 3). In contrast, after additionally adjusting for smoking, BMI, and dyslipidemia, higher levels of TAT_{volume}, VAT_{volume} as well as the VAT/SAT_{volume} ratio remained significantly higher in subjects with prediabetes and diabetes compared to controls (all <0.016), while the association of SAT_{volume} with glycemic state attenuated and became non-significant (all $p \geq 0.14$).

Association with BMI and Waist Circumference

Figure 4 displays the correlation beteween the absolute fat depot volumes and the VAT/SAT_{volume} ratio with rising BMI or waist circumference in controls as well as subjects with impaired glucose metabolism. With rising BMI and waist circumference an increase of VAT_{volume} and SAT_{volume} was detected in all subgroups. The increase of SAT_{volume} with rising BMI and waist circumference was similar in all subgroups whereas there was a stronger incresase of VAT_{volume} in controls as compared to subjects with prediabetes and diabetes ($r=0.64$ for controls vs. $r=0.34$ and $r=0.62$ in subjects with prediabetes and diabetes, respectively).

Figure 5 displays the marginal effect of glycemic status on the VAT/SAT_{volume} ratio for multiplicative interactions with BMI and waist circumference. The marginal effect reached statistical significance for a BMI up to 29.5 kg/m^2 and 31 kg/m^2 in subjects with prediabetes and diabetes, respectively ($p < 0.05$). Similarly, the marginal effect of glycemic status on the VAT/SAT_{volume} ratio reached statistical significance in the range of a waist circumference of 65-101 cm. The analysis of the absolute fat volumes VAT_{volume} and SAT_{volume} showed a decreasing marginal effect of diabetes on VAT_{volume} in the range of a BMI of 19-34 kg/m^2 and of prediabetes in the range of a BMI of 19.5-31 kg/m^2. An increasing marginal effect of glycemic status on SAT_{volume} was found, which did not reach statistical significance.

DISCUSSION

In this study, including adult individuals without known cardiovascular disease from the general population, we found a very strong correlation between volumetric and single-sliced measurements of VAT and SAT and its ratio. Increased MRI-based VAT_{volume} and VAT/SAT_{volume} ratios were associated with prediabetes and diabetes, independent of cardiometabolic confounders. Among measurements, VAT_{volume} was stronger related to prediabetes or diabetes as compared to TAT_{volume}, BMI, or waist circumference, while the association of SAT_{volume} was not independent of potential confounders. Furthermore, we found an attenuated association of VAT/SAT_{volume} ratio with glycemic state in obese subjects with high BMI or waist cirumference, possibly dominated by the variation of VAT_{volume} in obese subjects.

Similar to previous research, we found strong correlations between volumetric and single-sliced assessment of VAT and SAT as well as the VAT/SAT ratios. Schwenzer et al. found similarly high correlations between single slices and volumetric measurements of the several adipose tissue departments (28). Furthermore, in a study with morbidly obese patients, Schaudinn et al. found a strong correlation between volumetric VAT and sliced-based VAT, independent of the number of slices assessed (37). However, our data also indicate that a volumentric measurements may provide a slightly higher discriminaroty power, particulary in subjects with higher BMI. In contrast to these earlier efforts, our sample was drawn from a large European general population without prior cardiovascular disease and comprised subjects with impaired glycemic state as well as controls, thus, allows for higher generalizability. As such, while we confirm that a single-slice based quantification of adipose tissue depots represent a reliable alternative for risk stratification in larger cohorts, further more outcome realted research will be necessary.

Despite the strong association among these quantitative parameters, there is early evidence that VAT_{volume} is a stronger predictor for metabolic disease and cardiovascular risk factors as compared to SAT (5, 12). Also, the VAT/SAT ratio seems to be a proxy for cardiometabolic risk, independent of VAT or absolute fat volumes (31, 38). Our results confirm these early findings, as we found a significant association of VAT, SAT and TAT as well as the VAT/SAT ratio with prediabetes and diabetes. Futhermore, our results indicate that VAT_{volume} as well as the VAT/SAT_{volume} ratio is strongly associated with diabetes and prediabetes state, independent of cardiometabolic risk factors, such as age, sex, hypertension, BMI, smoking and dyslipidemia. In contrast, the association of SAT and glycemic state attenuated after adjusting for these confounders. Furthermore, in contrast to VAT_{volume}, the SAT_{volume} as well as TAT_{volume} did not exceed a weak correlation with the VAT/SAT_{volume} ratio, potentially indicating a stronger influence of VAT_{volume} on the composition of body fat depots. We also found a stronger association of VAT_{volume} with increased risk of prediabets and diabetes compared to SAT_{volume}, VAT/SAT_{volume} ratio, BMI and waist circumference. In a large sample drawn from the Framingham Heart Study, including 3,001 participants without prior cardiovascular diseases, VAT was more strongly associated with adverse metabolic risk profile as compared to SAT; however, their measurements were performed on CT (5). Similary to our MR-based approach, in a large cohort of Chinese adults, Tang et al. found a higher association of VAT with increased risk of prediabetes (12). However, the role of SAT in cardiometabolic risk is still controversial, as previous studies found an inverse assoication of SAT with insulin resistance in obese subjects (39). As such, our results confirm the strong role of VAT_{volume} in predicting cardiovascular risk and potentially adverse outcome beyond SAT_{volume} in an European cohort.

This finding is mirrored for the role of VAT/SAT ratio. Previously, Kaess et al. found a significant correlation between VAT/SAT ratio and cardiometabolic risk factors, independent of BMI and absolute VAT (31). In a retrospective cohort including participants without known cardiovascular disease from Europe, Ladeiras-Lopes et al. found that CT-based VAT/SAT ratio was, in contrary to the absolute fat volumes of VAT and SAT, an independent risk factor for cardiovascular events and death (38). Our results suggest a stronger predictive value for absolute VAT_{volume}, while the VAT/SAT ratio remained an independently association of potential confounders. Further, outcome-based research is clearly needed to elucidate the most predictive parameter of VAT for risk stratification.

Interestingly, our results demonstrate an interaction effect between BMI and waist circumference and prediabetes and/or diabetes state, as the association between the VAT/SAT_{volume} ratio attenuated with higher BMI or waist circumference. Specifically, the relationship between absolute fat volumes (VAT_{volume} and SAT_{volume}) and BMI or waist circumference was characterized by a stronger increase of VAT_{volume} in controls as compared to subjects with impaired glucose metabolism, whereas SAT_{volume} increased similarly between the subgroups. Thus, the VAT/SAT_{volume} ratio decreased with increasing BMI or waist circumference in subjects with impaired glucose metabolism, which was the opposite in controls. Furthermore, in contrary to SAT_{volume} we found a strong correlation between VAT/SAT_{volume} ratios with VAT_{volume} measurement, indicating a stronger influence of VAT_{volume} compared to SAT_{volume}. These findings may suggest that the association of glycemic status with VAT/SAT_{volume} ratio is less pronounced in subjects with higher BMI or waist circumference and consequently limit the value of VAT/SAT_{volume} ratios for the risk stratification in these obese subjects du to the varying VAT_{volume} in obese patients. However, further confirmatory research also in other cohorts is clearly warranted.

Our study has several limitations. The small sample size as well as the inclusion of mainly middle aged, caucasian subjects limit the generalizability of our results and reported associations may differ according to ethnicity when comparing with other cohorts. Focusing on the relation of the fat depots in obese patients, generalizabilitiy is limited due to the fact of the small number of subjects with high levels of BMI and waist cirucmference. However, our study population represent a representative sample from a western European population. Furthermore, the observational cross-sectional design of our study precludes definite causal interferences and more large-scale studies are warranted.

In conclusion, our results demonstrate that there is a strong correlation between the different parameters of fat deposition, including SAT, VAT and VAT/SAT ratios derived from area-based and volumentric MRI. Among them, elevated VAT_{volume} and VAT/SAT_{volume} ratio are associated with prediabetes and diabetes, above and beyond known cardiovascular risk factors and independent of single-sliced or volumetric quantification on MRI. However, VAT/SAT_{volume} ratios apear to be more dependent on VAT_{volume} as compared to SAT_{volume} or TAT_{volume}. In obese subjects with elevated BMI and/or waist circumference, the VAT/SAT_{volume} ratio may be of limited value due to present interaction effects. Thus, quantification of VAT represents a reproducable and reliable biomarker for cardiometabolic risk. Further confirmatory research especially in large cohort-studies is warranted.

TABLES

Table 1: Demographic characteristics and cardiovascular risk factors of our study population.

Variable	**All** **N = 384**	**Control** **N = 235 (61.2%)**	**Prediabetes** **N = 97 (25.3%)**	**Diabetes** **N = 52 (13.5%)**
Age, years	56.2 ± 9.2	54.0 ± 8.7	58.5 ± 8.9	62.1 ± 8.3
Male gender	223 (58.1%)	121 (51.5%)	63 (64.9%)	39 (75.0%)
Weight, kg	82.5 ± 15.9	78.6 ± 15.4	88.8 ± 13.4	88.1 ± 17.3
Height, cm	171.7 ± 9.7	171.6 ± 10.3	172.2 ± 9.4	171.5 ± 7.8
BMI, kg/m^2	27.9 ± 4.7	26.6 ± 4.2	30.0 ± 4.5	29.9 ± 4.9
Waist circumference, cm	98.0 ± 13.8	93.4 ± 12.5	104.4 ± 11.7	106.9 ± 14.1
Waist-To-Hip-Ratio	0.9 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	1.0 ± 0.1
Hypertension	128 (33.3%)	49 (20.9%)	43 (44.3%)	36 (69.2%)
HbA1c, %	5.6 ± 0.7	5.3 ± 0.3	5.6 ± 0.3	6.7 ± 1.3
HDL, mg/dl	61.9 ± 17.7	65.1 ± 17.9	58.7 ± 14.3	53.8 ± 18.9
LDL, mg/dl	139.4 ± 32.6	138.2 ± 31.5	146.1 ± 30.3	132.8 ± 39.4
Triglycerides, mg/dl	131.5 ± 85.8	107.5 ± 64.3	152.0 ± 82.8	201.3 ± 122.3
Total cholesterol, mg/dl	217.7 ± 36.2	215.7 ± 35.6	225.5 ± 31.5	212.6 ± 44.7
Smoking				
Never-smoker	141 (36.7%)	92 (39.1%)	34 (35.1%)	15 (28.8%)

Ex-smoker	165 (43.0%)	91 (38.7%)	45 (46.4%)	29 (55.8%)
Smoker	78 (20.3%)	52 (22.1%)	18 (18.6%)	8 (15.4%)

Data are presented as arithmetic means ± standard deviations (continuous variables) or counts and percentages (categorical variables). LDL=low-density lipoprotein, HDL=high-density lipoprotein.

Table 2. Difference of visceral and subcutaneous adipose tissue between subjects with prediabetes, diabetes, and healthy controls.

	All N = 384	**Controls** N = 235	**Prediabetes** N = 97	**p-value***	**Diabetes** N = 52	**p-value****
Body Adipose Tissue						
TAT_{volume}, l	12.6 ± 5.5	10.7 ± 4.7	15.3 ± 5.3	< 0.001	16.1 ± 5.4	< 0.001
VAT_{volume}, l	4.5 ± 2.7	3.4 ± 2.3	5.8 ± 2.4	< 0.001	6.9 ± 2.5	< 0.001
SAT_{volume}, l	8.1 ± 3.7	7.3 ± 3.2	9.6 ± 4.2	< 0.001	9.2 ± 3.8	0.001
ratio VAT/SAT_{volume}	0.59 ± 0.33	0.49 ± 0.29	0.68 ± 0.34	< 0.001	0.82 ± 0.34	< 0.001

* against controls, ** against controls. P-values are Bonferroni corrected for the repeated comparison to the control group. TAT=total adipose tissue; SAT=subcutaneous adipose tissue; VAT=visceral adipose tissue

Table 3: Association of glycemic status to body adipose tissue after adjustment for potential confounders.

Adjusted for age, sex and BMI						
	Prediabetes			**Diabetes**		
	β-coefficient	95%-CI	p-value	β-coefficient	95%-CI	p-value
TAT_{volume}, l	1.08	[0.46, 1.69]	< **0.001**	1.80	[1.01, 2.58]	< **0.001**
VAT_{volume}, l	0.76	[0.37, 1.16]	< **0.001**	1.50	[0.99, 2.01]	< **0.001**
SAT_{volume}, l	0.32	[-0.10, 0.73]	0.14	0.30	[-0.23, 0.83]	0.271
Ratio VAT/SAT_{volume}	0.10	[0.04, 0.15]	< **0.001**	0.15	[0.08, 0.22]	< **0.001**
Adjusted for age, sex, smoking, body mass index, hypertension, HDL, LDL and triglycerides						
	Prediabetes			**Diabetes**		
	β-coefficient	95%-CI	p-value	β-coefficient	95%-CI	p-value
TAT_{volume}, l	0.82	[0.21, 1.44]	**0.009**	1.19	[0.34, 2.04]	**0.006**
VAT_{volume}, l	0.52	[0.14, 0.91]	**0.008**	0.87	[0.34, 1.40]	**0.001**
SAT_{volume}, l	0.30x	[-0.12, 0.72]	0.166	0.32	[-0.26, 0.90]	0.281

Ratio VAT/SAT$_{volume}$	0.07	[0.02, 0.13]	**0.008**	0.09	[0.02, 0.16]	**0.02**

Results from linear regression model. CI, confidence interval. TAT=total adipose tissue; SAT=subcutaneous adipose tissue; VAT=visceral adipose tissue.

FIGURE LEGENDS

Figure 1: MRI-based assessment of adipose tissue depots in a 42-year old male control (A-C; VAT_{volume} 2.8 l, SAT_{volume} 5.8 l, VAT_{area} 89.8 cm^2, SAT_{area} 259.4 cm^2) and an obese, 57-year old male with prediabetes (D-F; VAT_{volume} 9.1 l, SAT_{volume} 10.8 l, VAT_{area} 302.3 cm^2, SAT_{area} 332.2 cm^2). The volumes of the different adipose tissue depots were measured automatically from the diaphragm to the femoral head by employing an inhouse algorithm (B-C and E-F). VAT_{area} and SAT_{area} are derived from a single slice on the level of the umbilicus (A and D). (*red area*=VAT; *yellow area*=SAT).

Figure 2. Scatter plots demonstrating the correlation between single-sliced and volumetric assessment of VAT and SAT determined by MRI. SAT=subcutaneous adipose tissue; VAT=visceral adipose tissue.

Figure 3. Scatter plots demonstrating the correlation between VAT_{volume}, SAT_{volume} and TAT_{volume} with the respective VAT/SAT_{volume} ratio determined by MRI. SAT=subcutaneous adipose tissue; VAT=visceral adipose tissue, TAT=total adipose tissue.

Figure 4. Association of adipose tissue depots SAT_{volume} and VAT_{volume} as well as the VAT/SAT_{volume} ratio obtained with increasing BMI and waist circumference. SAT=subcutaneous adipose tissue; VAT=visceral adipose tissue; BMI=body mass index.

Figure 5. Marginal effects of glycemic status on the ratio of VAT/SAT_{volume} for multiplicative interactions with BMI (left) and waist circumference (right). Displayed are the marginal effects of prediabetes (solid line, dark grey) and diabetes (solid line, light grey) and the

respective 95% confidence interval for a grid of possible values of BMI (range in data: 18.1 – 43.2 kg/m^2) and waist circumference (range in data: 66.4 – 144.8 cm). The arithmetic mean is indicated by a dotted line.The dashed line indicates the line of no effect.

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Figure1

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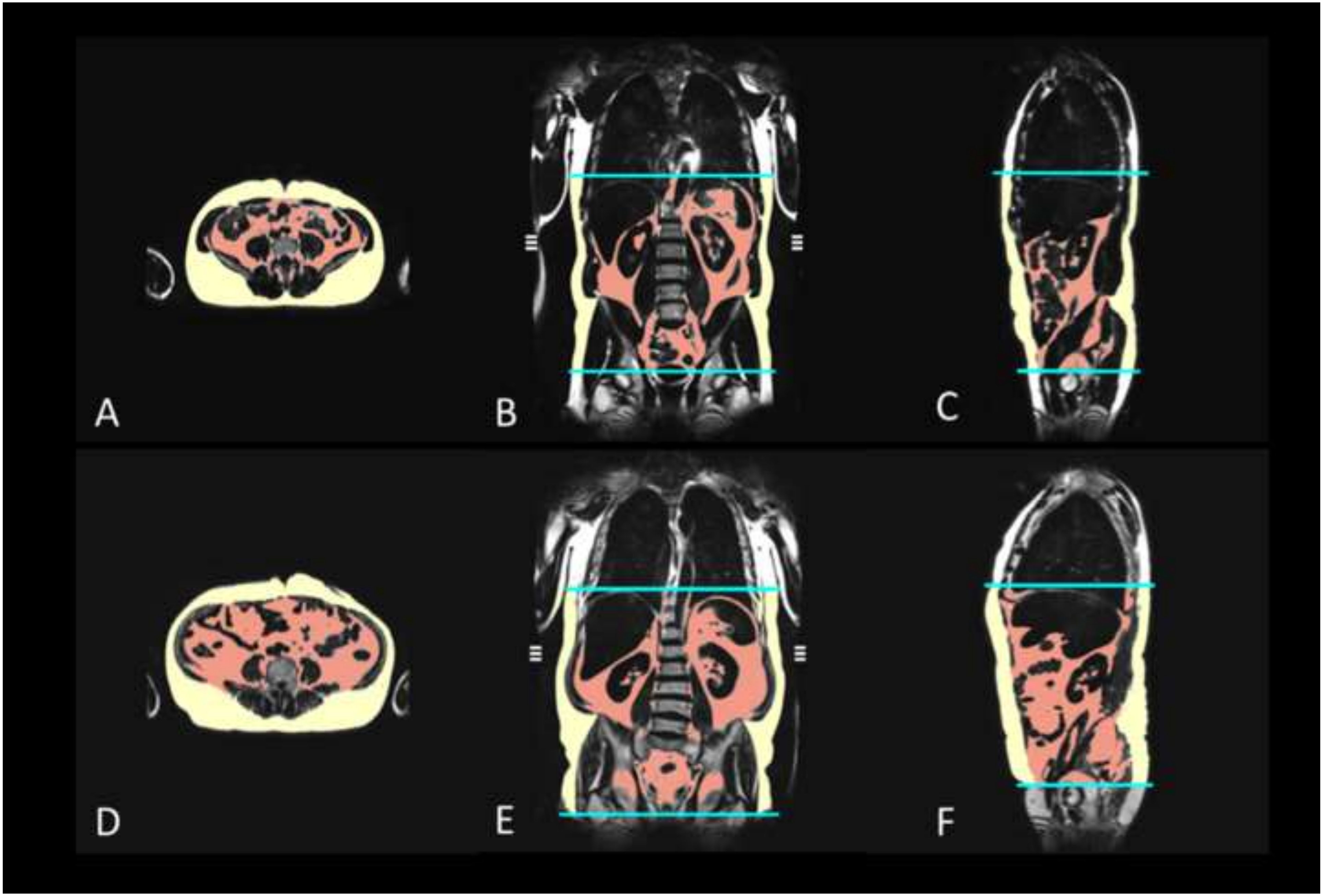

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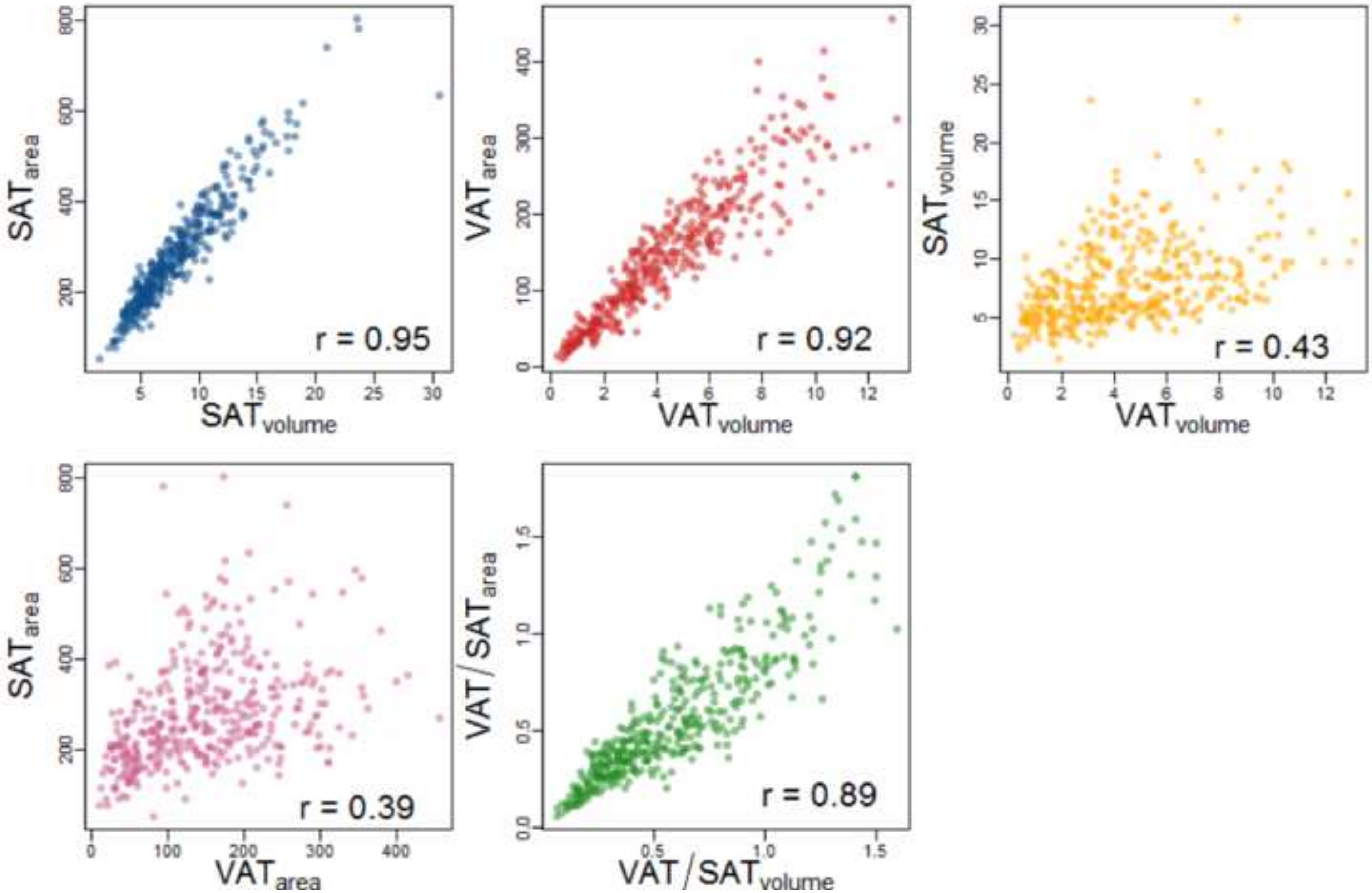

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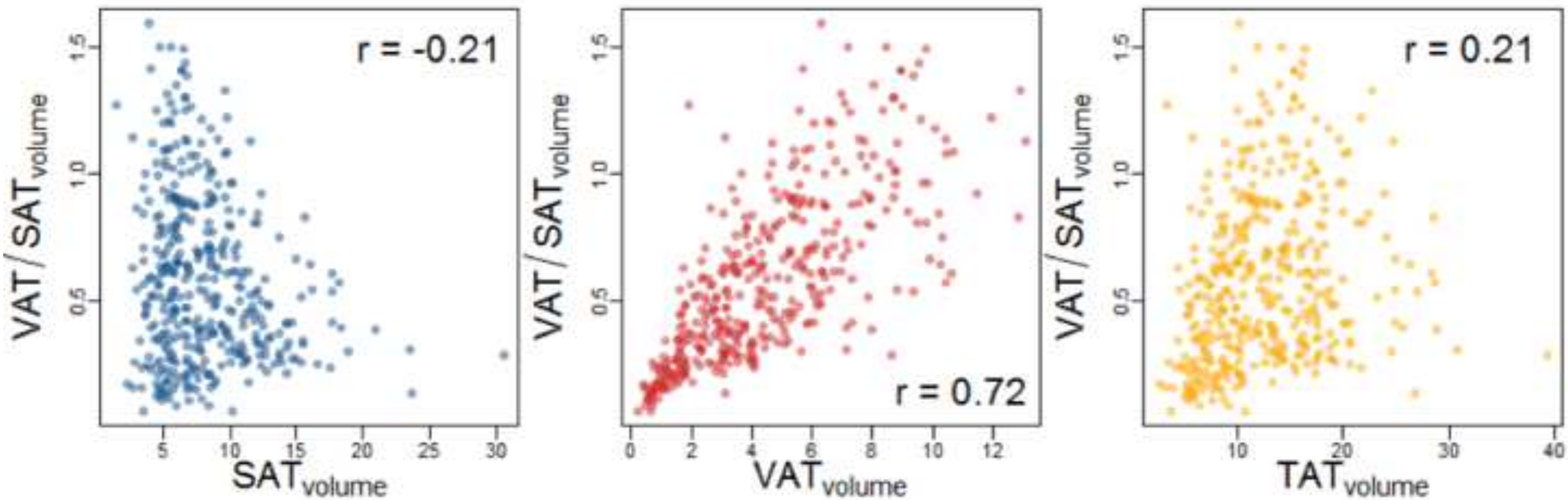

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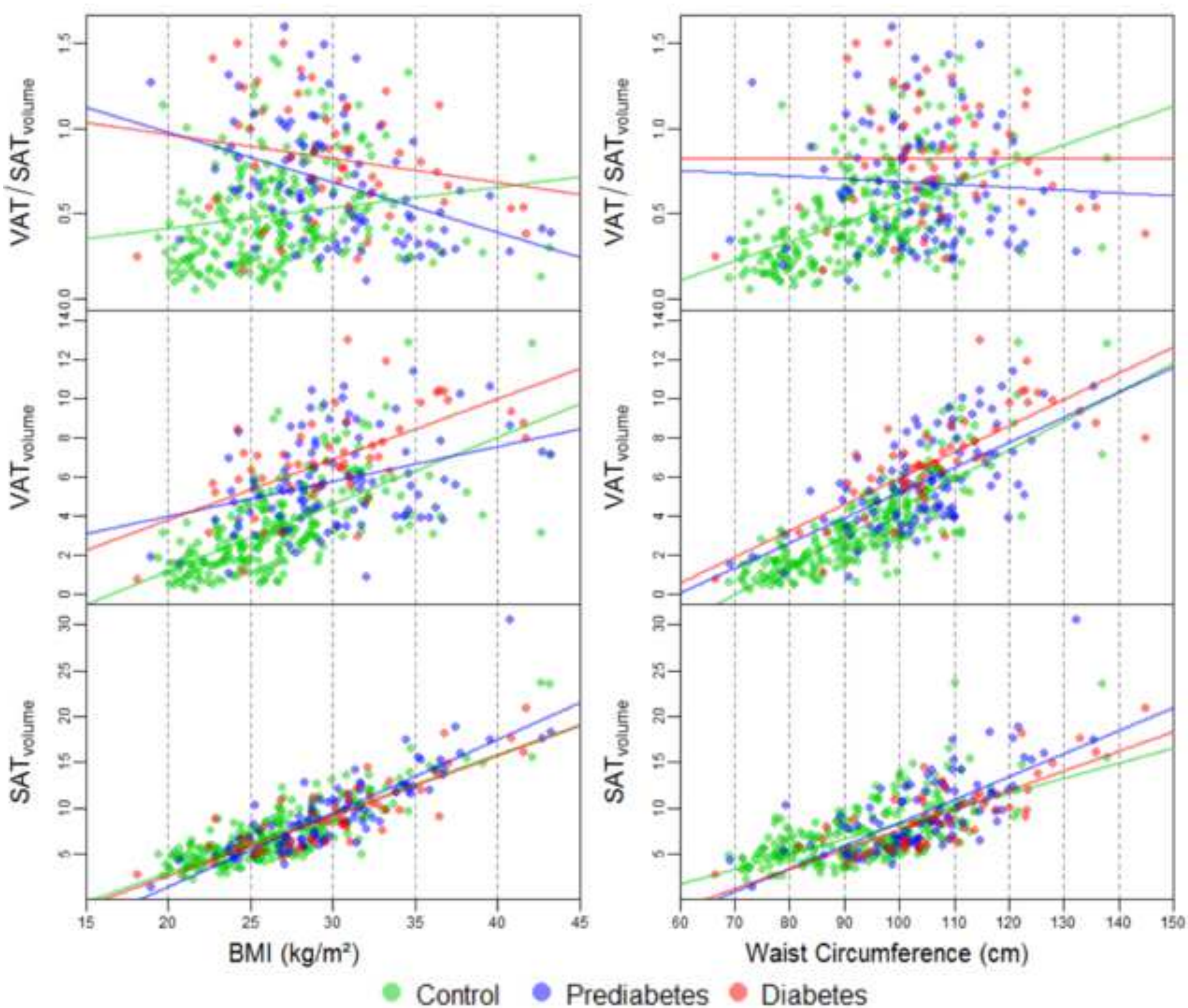

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