Assessment of the Degree of Abdominal Myosteatosis by Magnetic Resonance Imaging in Subjects with Diabetes, Prediabetes and Healthy Controls from the General Population

Short Title: Myosteatosis by MRI

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Conflicts of interest and sources of funding: This study was funded by the German Research Foundation (DFG, Bonn, Germany), the German Centre for Cardiovascular Disease Research (DZHK, Berlin, Germany) and the Centre for Diabetes Research (DZD e.V., Neuherberg, Germany). The KORA study was initiated and financed by the Helmholtz Zentrum München - German Research Center for Environmental Health, which is funded by the German Federal Ministry of Education and Research (BMBF) and by the State of Bavaria

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ABSTRACT

Objectives: Intra- and intermyocellular lipid deposition and adipose tissue are part of glucose homeostasis and insulin resistance; however, their role in type 2 diabetes mellitus (T2DM) remains unclear. We assessed differences in the degree of abdominal myosteatosis among subjects with T2DM and prediabetes.

Materials and Methods: Asymptomatic subjects from the general population were classified as subjects with T2DM, prediabetes or healthy controls and underwent multi-echo Dixon magnetic resonance imaging (MRI) (TR 8.90ms, six echo times, flip-angle 4°). Abdominal myosteatosis was quantified as proton-density fat-fraction ($PDFF_{muscle}$) by a standardized segmentation-algorithm. Cardiometabolic risk factors were prospectively obtained in a comprehensive health assessment and visceral and subcutaneous adipose tissue (VAT and SAT) were quantified semi-automatically. Uni- and multivariate quantile regression were used to examine associations.

Results: Among 349 included subjects (mean age: 56.0±8.0years, 56.7% males), 45 were classified as subjects with T2DM and 84 with prediabetes (12.9% and 24.1%; respectively). Median $PDFF_{muscle}$ was significantly higher in subjects with T2DM and prediabetes compared to healthy controls (13.1% (IQR10.5-16.6%); 11.1% (IQR8.9-15.0%) and 10.1% (IQR7.5-13.3%); respectively, $p<0.001$). The observed differences were independent of age and gender (all $p<0.002$) but attenuated after adjustment for BMI (β:-0.02, 95%CI:-1.49-1.44, p=0.974; β:0.47, 95%CI:-0.91-1.86, p=0.506; prediabetes and T2DM, respectively). This effect was attributable to VAT, which remained independently associated with $PDFF_{muscle}$ after full adjustment (β:0.01, 95%CI:0.01-0.02, p=0.002).

Conclusions: There are significant differences in the degree of abdominal myosteatosis between subjects with T2DM, prediabetes and healthy controls, that may be confounded by VAT. However, abdominal myosteatosis by MRI might serve as a cardiometabolic imaging-biomarker, specifically in the setting of impaired glucose metabolism.

Key Words: Myosteatosis, diabetes mellitus, cardiometabolic risk factors, skeletal muscle segmentation, magnetic resonance imaging.

INTRODUCTION

Diabetes mellitus (DM) as one of the most common metabolic disorders, affects more than 415 million people worldwide.[1] Furthermore, over 318 million people in the world are estimated to suffer from the precursor stage of DM, prediabetes[1], a condition with impaired glucose metabolism, which is highly likely to progress to an established type 2 DM (T2DM). Due to ongoing demographic transition and progressive aging of the population, the prevalence of both entities will further increase and DM-related comorbidities, long-term complications and DM-associated mortality will become a major healthcare burden.[1,2] Thus, further research on pathophysiological changes as potential risk factors specifically in the context of prevention as well as early diagnosis and treatment of asymptomatic subjects with impaired glucose metabolism in incident prediabetes and DM is needed.

A major risk factor for the development and progression of T2DM is the metabolic syndrome with its symptoms high fasting serum triglycerides, low high-density lipoprotein (HDL), elevated fasting plasma glucose and blood pressure as well as abdominal obesity.[3] Despite abdominal adipose tissue compartments, such as visceral and subcutaneous adipose tissue (VAT and SAT)[4], ectopic lipid deposits for example in liver or skeletal muscle play an important role in the pathophysiology of insulin resistance.[5–7] Since skeletal muscle is a major target organ of insulin, recent data suggest that changes in fat content, such as intermyocellular-intrafascial adipose tissue infiltration or intramyocellular lipid deposition, may be strong correlates of an impaired glucose homeostasis.[6–8] Furthermore, myosteatosis may be a potential mediator of development and progression of insulin resistance, cardiovascular risk factors and other DM-related comorbidities and complications.[2,8,9] Yet, it remains unclear whether myosteatosis is a causal mechanism or just a coincidental bystander in insulin resistance and T2DM. Thus, further research is needed to asses both, DM-

related change of myosteatosis and its pathophysiological relevance and clinical implications as a potential diagnostic and prognostic imaging-biomarker in impaired glucose metabolism.

Therefore, we systematically determined the degree of abdominal myosteatosis by a magnetic resonance imaging (MRI)-based, manual abdominal skeletal muscle segmentation in subjects with T2DM, prediabetes and healthy controls from a population-based cohort. Furthermore, we assessed associations with cardiometabolic risk factors as well as other adipose tissue compartments. We hypothesized that there are differences in the degree of abdominal myosteatosis, which are independently associated with impaired glucose metabolism and may therefore serve as imaging-biomarkers in cardiometabolic risk stratification.

MATERIALS AND METHODS

Ethics Statement

The study was approved by the local institutional review board of the Ludwig-Maximilian-University Munich. Written informed consent was obtained from all participants. All methods and analyses were carried out in accordance with the approved protocol and guidelines and all records were anonymized.

Study Design and Population

Subjects were derived from the KORA-FF4 study (2013-2014, n=2279), a 14-year follow-up study of the population-based Cooperative Health Research in the Region of Augsburg (KORA) survey S4 (1999-2001, n=4261) in Southern Germany. The design of the KORA studies has been described in detail previously.[10] 400 eligible subjects underwent whole-body MRI according to previously described inclusion and exclusion criteria.[10] A comprehensive health assessment was prospectively performed for all subjects to obtain potential covariates, such as diabetes status and other cardiometabolic risk factors.

Covariates

To determine the glycemic status, a 75g oral glucose tolerance test was performed for all subjects not yet being diagnosed with T2DM. According to the WHO-definition, subjects were classified as subjects with established T2DM (two-hour plasma glucose following a 75g oral glucose tolerance test (OGTT) ≥11.1mmol/l and/or fasting plasma glucose (FPG) ≥7.0mmol/l), as subjects with prediabetes with impaired fasting glucose (IFG) and/or impaired glucose tolerance (IGT) (OGTT 7.8-11.0mmol/l and/or FPG 5.6-6.9mmol/l) or healthy controls (OGTT <7.8mmol/l and/or FPG <5.6mmol/l).[11] The body mass index (BMI) was calculated as weight in kg divided by

body height squared in m^2. Hypertension was determined according to the WHO-definition as systolic blood pressure ≥140mmHg and/or diastolic blood pressure ≥90mmHg or current intake of antihypertensive medication.[12] Alcohol consumption and smoking status was classified by self-report as no alcohol at all (0g/day), moderate alcohol consumption (males: 0.1-39.9g/day, females: 0.1-19.9g/day) or heavy alcohol consumption (males: >40g/day, females: >20g/day) and never-smoker, ex-smoker and current (regular or sporadic) smoker. Regarding physical activity, subjects were categorized as physically active (regular physical activity ≥1h/week) or physically inactive (irregular physical activity <1h/week, almost no physical activity and no physical activity at all). Routinely intake of medication was generally categorized according to most recent guidelines. Statins, fibrates or other lipid-lowering medication were categorized as lipid-lowering medication, medication containing glucocorticoids or mineralocorticoids was categorized as systemic corticosteroids which were separated from non-steroidal anti-inflammatory drugs (NSAIDs, for example ASS100 or ASS300).

MR Imaging Protocol and Data Acquisition

MR examinations were performed in supine position on a 3-Tesla Magnetom Skyra (Siemens Healthineers, Erlangen, Germany) using an 18-channel body surface coil in combination with a table-mounted spine matrix coil. The complete imaging protocol as well as technical specificities have been described in detail elsewhere.[10]

For the determination of the degree of abdominal myosteatosis, skeletal muscle fat content was quantified using a T2*-corrected, multi-echo 3D-gradient-echo Dixon-based sequence (multi-echo Dixon) with the following parameters: time to repetition (TR) 8.90ms, time to echo (TEs) opposed-phase 1.23ms, 3.69ms and 6.15ms, TEs in-phase 2.46ms, 4.92ms and 7.38ms, flip angle 4°, readout echo bandwidth

1080Hz/pixel, matrix 256x256, slice thickness 4mm. Data were acquired during a single breath-hold of 15s. The post-processing algorithm using the Software MR LiverLab (Version VD13, Siemens Healthineers, Cary, USA) automatically calculated water- and fat-only images as DICOM-files from the original data of the six echos. The obtained fat signal-fraction maps are based on the signal ratio of fat to the summed signal of water and fat (proton-density fat-fraction) and corrected for confounding effects of T1- and T2*-decay, quantitatively coding the mean proton-density fat-fraction (PDFF) in degrees of grey values of each voxel (1 intensity value = 0.1% fat content).[13] Furthermore, coronal two-point Dixon gradient-echo (GRE) sequences (TR 4.06ms, TE 1.26ms and 2.49ms, flip angle 9°, slice thickness 1.7mm, isotropic in-plane resolution 1.7mm) were used for the identification of L3 vertebra on axial slices by cross-reference.

MR Image Analysis and Skeletal Muscle Segmentation

The DICOM-files of the fat signal-fraction maps were implemented into the commercially available Software OsiriX (V8.5.1, Pixmeo SARL, Bernex, Switzerland) on a dedicated, offline workstation. Skeletal muscle segmentation was performed on one axial slice at the level of the lower endplate of L3 vertebra. If L3 vertebra was not imaged, the most caudal possible axial slice was selected. Subjects with significant image artifacts on all levels were excluded from the analysis. If artifacts were limited to level L3, the next possible, cranial slice without artifacts was selected. Two independent observers blinded to the glycemic status and other information or clinical covariates of the subjects performed image analysis and skeletal muscle segmentation. The complete segmentation procedure of one data set took on average 5 minutes.[14]

The applied manual skeletal muscle segmentation algorithm as well as abdominal myosteatosis itself were shown to be highly reproducible with excellent inter- and intraobserver reproducibility for all included muscle compartments (ICC 0.94-1.0, -0.2±0.5%, -2.6±6.4%; ICC 0.96-1.0, 0.0±0.4% 0.4±3.8%; respectively). Furthermore, measurement variabilities were independent of potential confounders such as age, gender, BMI, body height, VAT and skeletal muscle cross-sectional area (ICC 0.93 to 1.0).[14]

Abdominal Myosteatosis ($PDFF_{muscle}$)

The degree of abdominal myosteatosis was determined as mean skeletal muscle fat content ($PDFF_{muscle}$) in % using an anatomical landmark-based, manual segmentation of both the right and left psoas major muscle, the quadratus lumborum muscle, the autochthonous back muscles (containing the erector spinae muscles and the spinotransverse muscles) and the rectus abdominis muscle at the level of the lower endplate of L3 vertebra (**Figure 1**). The validity and reproducibility as well as details of this segmentation approach have been described previously.[13–15] In brief, each muscle compartment was manually segmented according to dedicated and standardized, anatomical landmarks. To avoid partial volume effects of surrounding adipose tissue, the regions of interest (ROIs) did not comprise the complete muscle cross-sectional area but were drawn a few voxels smaller concentrically. Thus, surrounding, extramyocellular-extramyofascial adipose tissue was excluded in order to only quantify intra- and intermyocellular-intrafascial lipids and adipose tissue.

VAT and SAT

VAT and SAT as abdominal adipose tissue compartments were segmented and quantified in cm^2 by a semi-automated algorithm based on fuzzy-clustering on one

axial slice at the level of the umbilicus. Therefore, axial slices with a slice thickness of 5mm were reconstructed based on 3D VIBE-Dixon sequences, which were assessed in coronal direction.[16,17]

Statistical Analysis

Baseline characteristics are presented as median with 1st and 3rd quartile (interquartile range (IQR)) for continuous variables and absolute counts with percentages for categorical variables. Differences in median values or counts between subjects with T2DM, prediabetes and healthy controls were assessed by Kruskal-Wallis equality-of-populations rank test (quantitative data) or $\chi 2$-test (qualitative data). Correlations of $PDFF_{muscle}$ with cardiometabolic risk factors (for example VAT, SAT, age and BMI) were evaluated by scatter plots and Spearman's rho correlation coefficients. Associations of the glycemic status and $PDFF_{muscle}$ were determined by median regression adjusted for further cardiometabolic risk factors. A p-value of <0.05 was considered to indicate statistical significance. Statistical analysis was performed using R V3.4.1 (R Core Team, www.r-project.org, 2017).

RESULTS

Study Population

Among 400 subjects, 25 (6.3%) were excluded due to insufficient image quality or because they did not successfully complete the imaging protocol and 26 subjects (6.5%) were subsequently excluded because of missing values in any of the covariates. Thus, a total of 349 subjects comprised the study cohort. There were no significant differences regarding demographics between included and excluded subjects. Detailed characteristics of the study population are provided in **Table 1**. Included subjects were predominantly middle-aged men (median age: 56.0years, IQR 48.0-64.0years; male gender: 56.7%). Regarding glucose tolerance, 45 subjects were classified with established T2DM, 84 as prediabetics with IFG and/or IGT and 220 as healthy controls (12.9%, 24.1% and 63.0%, respectively).

In general, subjects with T2DM and prediabetes had a higher prevalence of cardiometabolic risk factors being older and more likely male, having a higher BMI as well as systolic and diastolic blood pressure and more severe dyslipidemia (all $p<0.049$). In comparison to healthy controls, subjects with T2DM and prediabetes had a significantly higher amount of VAT and SAT and were significantly less physically active. They were more likely under regular medication, such as lipid-lowering medication and non-steroidal anti-inflammatory drugs (all $p<0.005$).

Abdominal Myosteatosis

Results of the $PDFF_{muscle}$-measurements are provided in **Table 2**. Overall, median $PDFF_{muscle}$ of all abdominal skeletal muscle compartments in all included subjects was 10.7% (IQR 8.2-14.0%). There were significant differences in $PDFF_{muscle}$ with respect to the different muscle compartments, being highest in the autochthonous back muscles and lowest in the quadratus lumborum muscle ($PDFF_{autochthonous\ back}$

$_{muscles}$ 16.2% (IQR 11.4-21.5%), $PDFF_{rectus\ abdominis}$ 12.2% (IQR 7.8-19.0%), $PDFF_{psoas\ major}$ 6.5% (IQR 5.1-8.9%) and $PDFF_{quadratus\ lumborum}$ 6.0% (IQR 4.0-8.4%); respectively, all $p<0.05$). Mean $PDFF_{muscle}$ was significantly higher in subjects with T2DM and prediabetes compared to healthy controls (13.1% (IQR 10.5-16.6%), 11.1% (IQR 8.9-15.0%) and 10.1% (IQR 7.5-13.3%); respectively, $p<0.001$) (**Figure 2**). Specifically, differences in $PDFF_{muscle}$ were statistically significant for the psoas major muscle and the autochthonous back muscles ($p<0.001$), whereas no statistical significant difference could be found for the quadratus lumborum muscle and the rectus abdominis muscle ($p>0.06$) (**Table 2**).

Predictors of Abdominal Myosteatosis

In univariate analysis, age, gender, BMI, T2DM and elevated plasma glucose levels, hypertension as well as VAT and SAT, were significantly and positively associated with $PDFF_{muscle}$ (**Figure 3** and **Table 3**). In contrast, $PDFF_{muscle}$ was independent of prediabetes, dyslipidemic changes of blood lipids, alcohol consumption, smoking status and physical activity while regular intake of lipid-lowering medication or non-steroidal anti-inflammatory drugs was negatively associated with $PDFF_{muscle}$ (**Table 3**).

Results of median regression, adjusting for potential general and obesity-associated confounders in multivariate analysis, are provided in **Table 4**. After adjustment for age and gender, T2DM but not prediabetes remained to be significantly and positively associated with $PDFF_{muscle}$. After further adjusting for BMI as another confounder, this association was attenuated. Similarly, adjustment for VAT attenuated the association of $PDFF_{muscle}$ with diabetes status. After full adjustment, only age and VAT remained independent and significant predictors of $PDFF_{muscle}$ (β: 0.09 (95%-CI: 0.04-0.13), $p<0.001$; β: 0.01 (95%-CI: 0.01-0.02), $p=0.002$; respectively) (**Table 4**).

DISCUSSION

Given the still ambiguous pathophysiological and clinical role of skeletal muscle in general and specifically myosteatosis in insulin resistance and T2DM, we systematically determined the degree of abdominal myosteatosis by MRI as a potential, cardiometabolic imaging-biomarker in subjects with T2DM, prediabetes and healthy controls in a population-based sample. Our results indicate that there are significant differences in the degree of psoas major- and autochthonous back muscle-myosteatosis regarding the glycemic status, with myosteatosis being lowest in healthy controls and highest in subjects with established T2DM. However, this association is dependent on other, cardiometabolic risk factors, such as gender and BMI, and may be confounded by age and VAT.

There are several studies analyzing the interaction of different adipose tissue compartments, insulin resistance and T2DM. Skeletal muscle has become an important topic of research in the context of DM recently, given its function as a major effector organ of insulin and its crucial role in local and global glucose homeostasis and insulin resistance.[9,18] Recent data suggest consistently, that both intramyocellular lipid deposits and intermyocellular-intrafascial AT infiltration in lower limb skeletal muscle are significantly correlated with an impaired glucose and insulin homeostasis in DM. Earlier findings by Goodpaster et al. and Brehm et al. demonstrated a positive association of intramyocellular lipids and insulin resistance in subjects with DM.[19,20] Similarly, several authors reported a positive association of insulin resistance and T2DM with intermyocellular adipose tissue.[5,7] Yet, it is still unknown whether myosteatosis is a causal mechanism or a just a coincidental bystander in the development and progression of insulin resistance and T2DM. Our study now provides new insights in a large cohort setting. Since previous findings by Kuk et al. demonstrated that abdominal and lower limb myosteatosis only show moderate

correlation[21], we studied the degree of abdominal myosteatosis by MRI extending former findings from the lower limb to abdominal skeletal muscle compartments. Furthermore, we gained former unreported results regarding the association of abdominal myosteatosis with the glycemic status and further, cardiometabolic risk factors.

In agreement with findings by Lee et al, our study showed a significantly higher degree of myosteatosis in the autochthonous back muscles compared to the psoas major muscle.[22] Furthermore, and in line with Miljkovic et al., we found a significant correlation of total abdominal skeletal muscle myosteatosis and the glycemic status in our study.[23] Regarding the different abdominal skeletal muscle compartments, our results specifically confirm this correlation for the psoas major muscle. However, in contrast to Miljkovic et al., we additionally found this correlation to be significant for the autochthonous back muscles, which was similarly described by Kaibori et al. in oncologic patients undergoing hepatocellular carcinoma resection.[24] Furthermore and unlike Miljkovic et al., we found that the association of total abdominal myosteatosis with the glycemic status is not independent but may rather be confounded by age and VAT. Thus, one could assume that total and compartment-specific abdominal myosteatosis may be just a coincidental bystander and not a causal mechanism in the development and progression of T2DM. However, further research in longitudinal studies is inevitably needed to strengthen this hypothesis as well as to further specify the observed differences with regard to function and fibre-type composition of the analyzed muscle compartments.

Besides these aspects, the assessment of the degree of abdominal myosteatosis might also be relevant in distinct patient populations, for instance in perioperative risk evaluation; however, its clinical implications will need to be determined. Yet, myosteatosis by MRI as used in this study can been seen as a

reliable, feasible and cost-effective imaging-biomarker for cardiometabolic risk stratification specifically regarding incident prediabetes and DM, given its validity, non-invasive and non-ionizing nature and its simple measurement procedure as a free byproduct in hepatic or abdominal MRI examinations.

Currently, there are still many different approaches trying to explain the mechanisms of the association of intra- and extracellular ectopic fat deposits in non-adipose tissue and glucose homeostasis, insulin resistance and T2DM. Since the correlation of such deposits for example in the heart, liver and pancreas with the diabetes status has been described inconsistently, one could assume that the pathophysiological and clinical implementations of ectopic adipose tissue depots differ depending on the organ affected and may vary by anatomical region resulting in different metabolic consequences.[25,26] Regarding myosteatosis, this could be an explanation for the unequal results regarding glycemic status correlations in different muscle compartments from our study. However, since the functional capacities of intra- and intermyocellular adipose tissue differ significantly[27], further studies are needed to elucidate the relationship of these adipose tissue-compartments in different skeletal muscle compartments with the glycemic status.

In this study, age and VAT were independent predictors of the degree of abdominal myosteatosis. There is consisting evidence, that abdominal adipose tissue compartments such as VAT and SAT are positively and significantly associated with diabetes status[28] and that VAT is a major risk factor for cardiometabolic disease.[29] In contrast to recent findings by Miljkovic et al., who reported an independent association of abdominal myosteatosis with insulin resistance[23,30], our results indicate a former unreported, rather VAT-dependent association of abdominal myosteatosis with the glycemic status, highlighting the central role of VAT in the pathophysiology of T2DM. We were further able to confirm that adipose tissue infiltration and lipid deposition in

abdominal skeletal muscle is age-dependent.[31] Indeed, our findings demonstrate that the degree of abdominal myosteatosis is independently and positively associated with age. These observations are in line with former studies regarding skeletal muscle of the lower extremity, for example by Delmonico et al. demonstrating that progressive aging is associated with an increase in the degree of myosteatosis regardless of changes in body weight.[32] Taken together, these findings suggest a direct local impact of aging in skeletal muscle composition regarding fat content.

In contrast, we could not find an independent association of abdominal myosteatosis and regular intake of lipid-lowering medication and non-steroidal anti-inflammatory drugs beyond VAT, whereas in univariate analysis both showed a significant and negative correlation with abdominal myosteatosis. Since these medications either influence circulating blood lipids but neither triglycerides, total cholesterol, LDL nor HDL was correlated with abdominal myosteatosis in univariate analysis, we assume that the protective effect lies beyond blood lipid modulation and may be based on the well-known respectively recently reported, anti-inflammatory effect of both potentially influencing local skeletal muscle metabolism.[33] This hypothesis is supported by the fact that the protective effect of non-steroidal anti-inflammatory drugs was more distinct compared to lipid-lowering medication.

Our study has several limitations. Due to the study design, metabolic groups were not fully matched with respect to age and gender in order to compensate for differences beyond abdominal myosteatosis. However, while multivariate analysis was used to adjust for potential confounders, our findings are limited by a relatively small sample size from the German population and the cross-sectional study design, thus requiring confirmation in larger, longitudinal cohort studies, for example the German National Cohort. Second, we did not compare our results to histopathology as the current gold standard for the quantification of fat content. However, former studies

have demonstrated the validity and reproducibility of the standardized, anatomical landmark-based, skeletal muscle fat quantification by multi-echo Dixon as used in this study.[19,27,[41]] Third, sample PDFF-measurements on one single axial slice at level L3 vertebra, as performed in this study, may not reproduce a heterogeneous distribution of myosteatosis within the entire muscle due to under-sampling. However, recent studies showed that level L4 and L3 vertebra are representative for the entire lumbar spine.[27,28] A single level-based PDFF-quantification may therefore represent a valid and cost-effective approach to the assessment of abdominal myosteatosis.

Conclusions

There are significant differences in the degree of abdominal myosteatosis between subjects with T2DM, prediabetes and healthy controls, which may be confounded by age and VAT in this population-based sample. However, abdominal psoas major- or autochthonous back muscle-myosteatosis might be used for cardiometabolic and musculoskeletal risk stratification as a reliable imaging-biomarker specifically in the context of early characterization of cardiometabolic disease states.

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FIGURES

Figure 1 Degree of abdominal myosteatosis by $PDFF_{muscle}$.

Higher **(A)** and lower **(B)** degrees of abdominal myosteatosis by an anatomical landmark-based, manual, abdominal skeletal muscle segmentation at level L3 vertebra. PDFF: proton-density fat-fraction.

Figure 2 Differences in the degree of total abdominal myosteatosis ($PDFF_{muscle}$) between subjects with T2DM, prediabetes and healthy controls.

PDFF: proton-density fat-fraction, T2DM: type 2 diabetes mellitus.

Figure 3 Correlations of $PDFF_{muscle}$ with VAT, SAT, age and BMI.

VAT: visceral adipose tissue, SAT: subcutaneous adipose tissue, PDFF: proton-density fat-fraction, BMI: body mass index. Grey: male, red: female

TABLES

Table 1 Demographics of the KORA study population.

Data are presented as median [1st Quartile, 3rd Quartile] for continuous variables and counts and percentages for categorical variables. P-values are from Kruskal-Wallis Test or χ^2Test, respectively. * Based on N = 345.

T2DM: type 2 diabetes mellitus, BMI: body mass index, VAT: visceral adipose tissue, SAT: subcutaneous adipose tissue.

Table 2 Skeletal muscle fat content as mean $PDFF_{muscle}$.

PDFF: proton-density fat-fraction, T2DM: type 2 diabetes mellitus.

Table 3 Univariate analysis of associations between demographics, cardiometabolic risk factors and $PDFF_{muscle}$.

β-coefficients derived from median regression. CI: confidence interval, T2DM: type 2 diabetes mellitus, BMI: body mass index, VAT: visceral adipose tissue, SAT: subcutaneous adipose tissue.

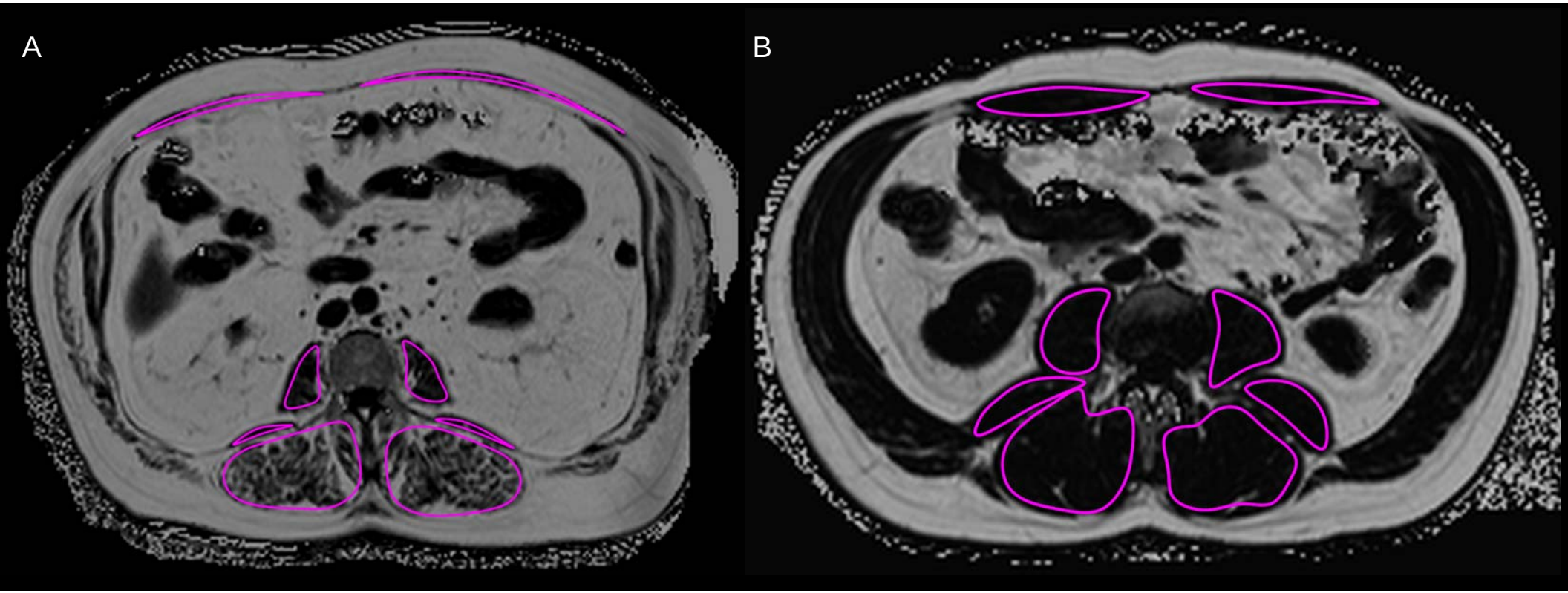
Table 4 Multivariate associations between demographics, cardiometabolic risk factors and $PDFF_{muscle}$.

Median regression including all variables in the table, outcome skeletal muscle. CI: confidence interval, T2DM: type 2 diabetes mellitus, BMI: body mass index, VAT: visceral adipose tissue, SAT: subcutaneous adipose tissue.

Acknowledgement Section:

Conflicts of interest and sources of funding: This study was funded by the German Research Foundation (DFG, Bonn, Germany), the German Centre for Cardiovascular Disease Research (DZHK, Berlin, Germany) and the Centre for Diabetes Research (DZD e.V., Neuherberg, Germany).

The KORA study was initiated and financed by the Helmholtz Zentrum München - German Research Center for Environmental Health, which is funded by the German Federal Ministry of Education and Research (BMBF) and by the State of Bavaria



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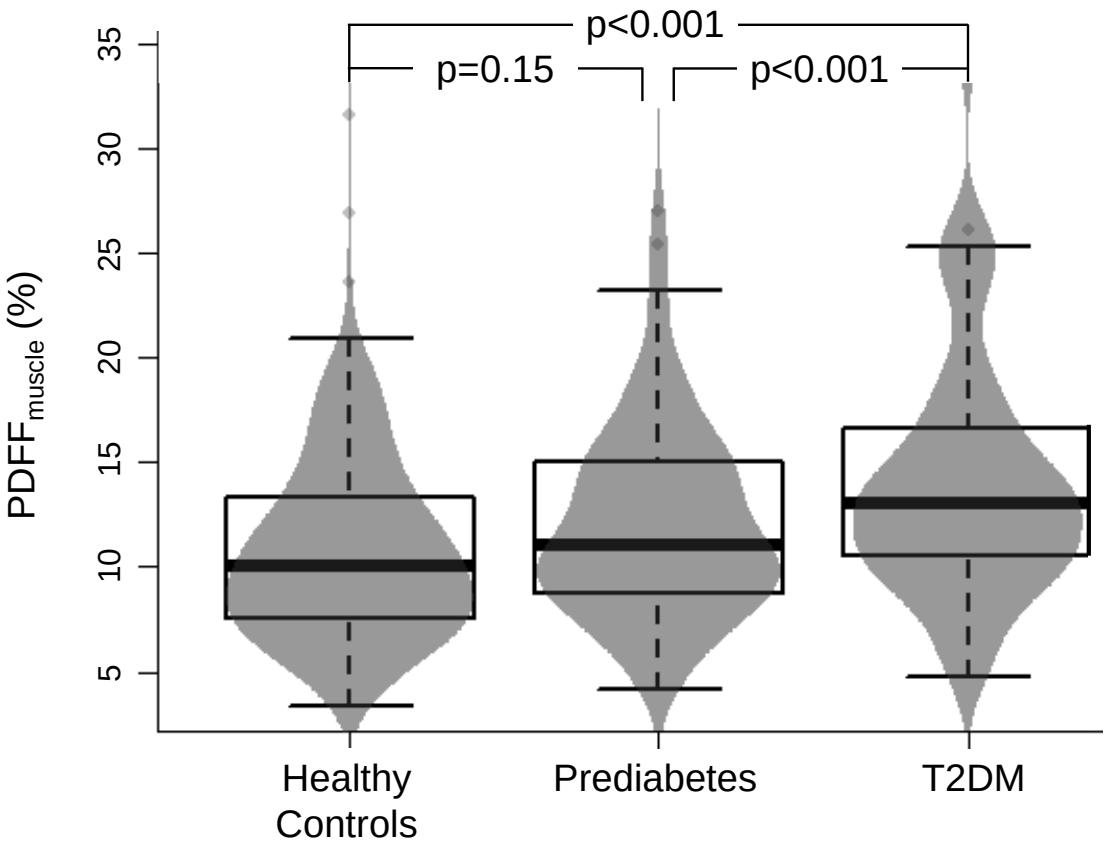

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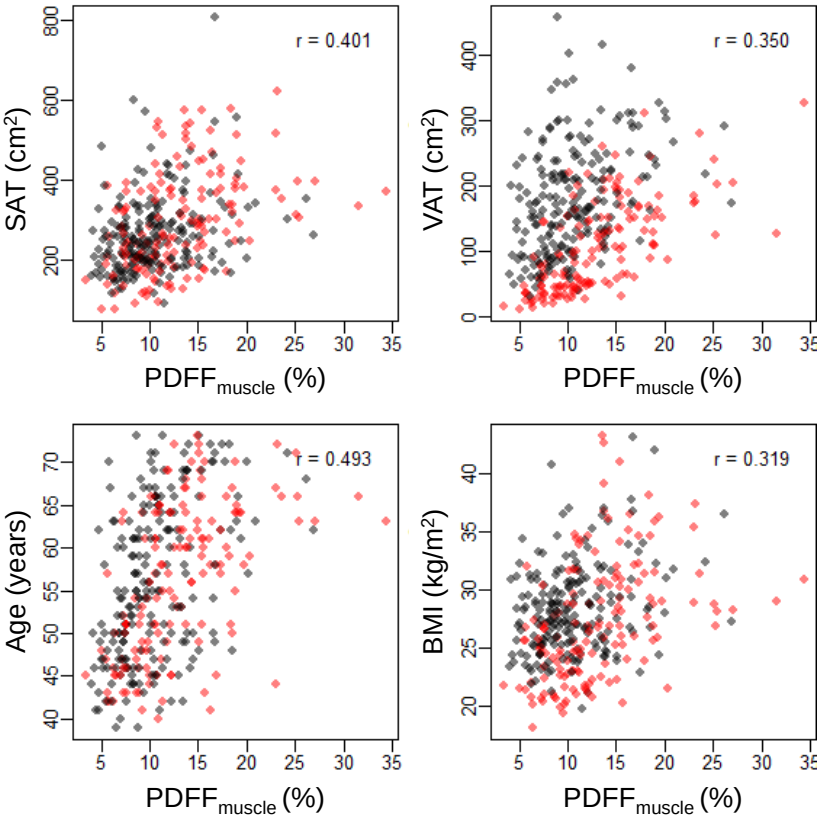

Figure 3 Correlations of $PDFF_{muscle}$ with VAT, SAT, age and BMI.

VAT: visceral adipose tissue, SAT: subcutaneous adipose tissue, PDFF: proton-density fat-fraction, BMI: body mass index. Grey: male, red: female

TABLES

Characteristics	All subjects	Healthy controls	Prediabetes	T2DM	p-value
N	**N = 349**	**N = 220 (63.0%)**	**N = 84 (24.1%)**	**N = 45 (12.9%)**	
Age (years)	56.0 [48.0, 64.0]	52.5 [47.0, 61.0]	58.0 [51.0, 65.2]	64.0 [58.0, 69.0]	0.005
Sex (male gender)	198 (56.7%)	111 (50.5%)	54 (64.3%)	33 (73.3%)	<0.001
BMI (kg/m^2)	27.2 [24.4, 30.6]	26.1 [23.7, 28.7]	29.4 [27.2, 32.0]	29.7 [26.8, 32.4]	<0.001
Hypertension	112 (32.1%)	46 (20.9%)	36 (42.9%)]	30 (66.7%)	<0.001
Smoking status					
Never-smoker	131 (37.5%)	87 (39.5%)	29 (34.5%)	15 (33.3%)	0.35
Ex-smoker	146 (41.8%)	84 (38.2%)	38 (45.2%)	24 (53.3%)	
Current smoker	72 (20.6%)	49 (22.3%)	17 (20.2%)	6 (13.3%)	
Alcohol consumption (g/day)					
no	85 (24.4%)	54 (24.5%)	17 (20.2%)	14 (31.1%)	0.33
moderate	198 (56.7%)	130 (59.1%)	46 (54.8%)	22 (48.9%)	
heavy	66 (18.9%)	36 (16.4%)	21 (25.0%)	9 (20.0%)	
Physically active	212 (60.7%)	143 (65.0%)	53 (63.1%)	16 (35.6%)	<0.001
HbA1c (%)	5.4 [5.3, 5.7]	5.4 [5.2, 5.5]	5.6 [5.4, 5.7]	6.5 [5.8, 6.9]	<0.001
Plasma-Glucose (mg/dl)	99.0 [92.0, 109.0]	95.0 [89.0, 100.0]	111.0 [101.0, 114.0]	139.0 [122.0, 152.0]	<0.001
Triglyceride levels (mg/dl)	105.0 [77.0, 152.0]	95.0 [70.0, 124.5]	131.6 [95.9, 182.2]	175.0 [108.4, 229.0]	<0.001
Total cholesterol (mg/dl)	217.0 [192.0, 240.0]	214.5 [190.0, 242.0]	225.0 [201.8, 242.5]	205.0 [182.0, 232.0]	0.049
HDL (mg/dl)	60.0 [49.0, 73.0]	62.0 [51.0, 77.0]	59.5 [49.9, 69.6]	49.0 [42.0, 62.0]	<0.001
LDL (mg/dl)	138.0 [117.0, 161.0]	137.0 [116.0, 162.0]	145.0 [127.2, 161.2]	133.0 [109.0, 150.0]	0.09
Medication					
Lipid-lowering medication	36 (10.3%)	14 (6.4%)	7 (8.3%)	15 (33.3%)	<0.001
Non-steroidal anti-inflammatory drugs	10 (2.9%)	3 (1.4%)	2 (2.4%)	5 (11.1%)	0.005
Corticosteroids	2 (0.6%)	1 (0.5%)	1 (1.2%)	0 (0.0%)	0.60
VAT (cm^2)	141.1 [79.7, 206.2]	100.4 [57.8, 154.3]	183.8 [138.0, 237.9]	217.4 [192.8, 289.9]	<0.001
SAT (cm^2)	256.3 [199.2, 334.9]	241.8 [184.5, 309.4]	292.6 [236.7, 391.3]	284.9 [211.4, 366.2]	<0.001

Table 1 Demographics of the KORA study population.

Data are presented as median [1st Quartile, 3rd Quartile] for continuous variables and counts and percentages for categorical variables.

P-values are from Kruskal-Wallis Test or χ^2Test, respectively. * Based on N = 345.

T2DM: type 2 diabetes mellitus, BMI: body mass index, VAT: visceral adipose tissue, SAT: subcutaneous adipose tissue.

	All subjects	Healthy controls	Prediabetes	T2DM	p-value
N	**N = 349**	**N = 220 (63.0%)**	**N = 84 (24.1%)**	**N = 45 (12.9%)**	
psoas major muscle	6.5 [5.1, 8.9]	6.0 [4.9, 8.2]	7.0 [5.5, 9.3]	8.7 [7.3, 10.7]	<0.001
quadratus lumborum muscle	6.0 [4.0, 8.4]	5.6 [3.9, 7.6]	6.2 [4.1, 8.7]	6.7 [4.2, 10.5]	0.07
autochthonous back muscles	16.2 [11.4, 21.5]	14.6 [10.5, 20.4]	16.5 [12.4, 22.8]	19.8 [14.3, 27.6]	<0.001
rectus abdominis muscle	12.2 [7.8, 19.0]	11.7 [7.4, 18.4]	13.8 [8.2, 19.7]	15.9 [9.5, 22.1]	0.09
mean skeletal muscle (all compartments)	10.7 [8.2, 14.0]	10.1 [7.5, 13.3]	11.1 [8.9, 15.0]	13.1 [10.5, 16.6]	<0.001

Table 2 Skeletal muscle fat content as mean $PDFF_{muscle}$.

PDFF: proton-density fat-fraction, T2DM: type 2 diabetes mellitus.

Predictor	**Estimate (Beta)**	**95%-CI**	**p-value**
Age (years)	0.24	[0.20, 0.28]	<0.001
Sex (female gender)	2.16	[0.78, 3.54]	0.002
BMI (kg/m^2)	0.29	[0.19, 0.40]	<0.001
Diabetes status			
Healthy control	Reference		
Prediabetes	0.99	[-0.37, 2.35]	0.15
T2DM	2.99	[1.40, 4.58]	<0.001
Hypertension	3.32	[1.87, 4.77]	<0.001
Smoking status			
Never-smoker	Reference		
Ex-smoker	0.34	[-0.82, 1.50]	0.57
Current smoker	-0.47	[-1.62, 0.68]	0.42
Alcohol consumption (g/day)			
No	Reference		
Moderate	0.04	[-1.22, 1.29]	0.95
Heavy	0.96	[-0.87, 2.79]	0.30
Physically active	-0.75	[-1.96, 0.46]	0.22
HbA1c (%)	1.30	[-0.12, 2.72]	0.07
Plasma glucose (mg/dl)	0.05	[0.01, 0.08]	0.01
Triglyceride levels (mg/dl)	0.01	[-0.00, 0.01]	0.12
Total cholesterol (mg/dl)	0.01	[-0.01, 0.03]	0.35
HDL (mg/dl)	0.01	[-0.01, 0.03]	0.40
LDL (mg/dl)	0.00	[-0.01, 0.02]	0.90
Medication			
Lipid-lowering medication	-3.67	[-6.14, -1.19]	0.004
Non-steroidal anti-inflammatory drugs	-5.95	[-10.08, -1.83]	0.005
VAT (cm^2)	0.02	[0.01, 0.03]	<0.001
SAT (cm^2)	0.02	[0.01, 0.02]	<0.001

Table 3 Univariate analysis of associations between demographics, cardiometabolic risk factors and $PDFF_{muscle}$.

β-coefficients derived from median regression. CI: confidence interval, T2DM: type 2 diabetes mellitus, BMI: body mass index, VAT: visceral adipose tissue, SAT: subcutaneous adipose tissue.

	Model 1 (age, gender, diabetes status)			Model 2 (Model 1 + BMI)			Model 3 (Model 1 + VAT)			Model 4 (fully adjusted)		
Predictor	**Estimate (Beta)**	**95%-CI**	**p-value**	**Estimate (Beta)**	**95%-CI**	**p-value**	**Estimate (Beta)**	**95%-CI**	**p-value**	**Estimate (Beta)**	**95%-CI**	**p-value**
Age (years)	0.20	[0.15, 0.26]	<0.001	0.21	[0.14, 0.28]	<0.001	0.17	[0.12, 0.23]	<0.001	0.09	[0.04, 0.13]	<0.001
Sex (female gender)	2.71	[1.64, 3.78]	<0.001	2.65	[1.69, 3.62]	<0.001	3.64	[2.81, 4.47]	<0.001	0.44	[-0.48, 1.36]	0.35
Diabetes status												
Prediabetes	1.01	[-0.30, 2.33]	0.13	-0.02	[-1.49, 1.44]	0.97	-0.34	[-1.57, 0.90]	0.60	-0.35	[-1.40, 0.70]	0.51
Diabetes (T2DM)	1.95	[0.57, 3.33]	0.006	0.47	[-0.91, 1.86]	0.51	-0.05	[-1.28, 1.19]	0.94	0.48	[-1.43, 2.39]	0.62
BMI (kg/m^2)	-	-	-	0.31	[0.17, 0.45]	<0.001	-	-	-	-0.09	[-0.26, 0.07]	0.27
VAT (cm^2)	-	-	-	-	-	-	0.02	[0.01, 0.03]	<0.001	0.01	[0.01, 0.02]	0.002
SAT (cm^2)	-	-	-	-	-	-	-	-	-	0.00	[-0.00, 0.01]	0.14
Hypertension	-	-	-	-	-	-	-	-	-	0.70	[-0.25, 1.65]	0.15
Smoking status												
Ex-smoker	-	-	-	-	-	-	-	-	-	0.41	[-0.39, 1.21]	0.31
Current smoker	-	-	-	-	-	-	-	-	-	0.32	[-0.66, 1.29]	0.53
Alcohol consumption (g/day)												
Moderate	-	-	-	-	-	-	-	-	-	0.03	[-0.90, 0.96]	0.95
Heavy	-	-	-	-	-	-	-	-	-	0.22	[-1.32, 1.76]	0.78
HbA1c (%)	-	-	-	-	-	-	-	-	-	-0.09	[-1.21, 1.03]	0.88
Plasma glucose (mg/dl)	-	-	-	-	-	-	-	-	-	0.01	[-0.04, 0.05]	0.72
Triglyceride levels (mg/dl)	-	-	-	-	-	-	-	-	-	0.00	[-0.01, 0.01]	0.75
Total cholesterol (mg/dl)	-	-	-	-	-	-	-	-	-	0.02	[-0.02, 0.07]	0.34
HDL (mg/dl)	-	-	-	-	-	-	-	-	-	-0.02	[-0.07, 0.03]	0.49
LDL (mg/dl)	-	-	-	-	-	-	-	-	-	-0.03	[-0.07, 0.02]	0.28

Table 4 Multivariate associations between demographics, cardiometabolic risk factors and $PDFF_{muscle}$.

Median regression including all variables in the table, outcome skeletal muscle. CI: confidence interval, T2DM: type 2 diabetes mellitus, BMI: body mass index, VAT: visceral adipose tissue, SAT: subcutaneous adipose tissue.