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ORIGINAL ARTICLE

Adipose tissue distribution in relation to insulin sensitivity and inflammation in Pakistani and Norwegian subjects with type 2 diabetes

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Abstract

Immigrants from South Asia to Western countries have a high prevalence of type 2 diabetes mellitus (T2DM) associated with obesity. We investigated the relationship between diabetes and adipose tissue distribution in a group of younger T2DM subjects from Norway and Pakistan. Eighteen immigrant Pakistani and 21 Norwegian T2DM subjects (age 29–45, 49% men) were included. They underwent anthropometrical measurements including bioelectrical impedance analysis, CT scans measuring fatty infiltration in liver and adipose and muscle tissue compartments in mid-abdomen and thigh, a euglycemic clamp, and blood samples for serum insulin and plasma glucose, adipokines and inflammation markers. Adipose tissue distribution was similar in Norwegians and Pakistanis. Pakistanis, but not Norwegians, showed a negative correlation between insulin sensitivity and visceral adipose tissue (VAT, $r_s = -0.704$, $p = 0.003$). Subcutaneous adipose tissue (SAT) correlated to leptin in both Pakistanis and Norwegians ($r_s = 0.88$, $p < 0.001$ and 0.67, $p = 0.001$). SAT also correlated to C-reactive protein (CRP) in the Pakistanis only ($r_s = 0.55$, $p = 0.03$), and superficial SAT to Interleukin-1 receptor antagonist (IL-1RA) in Norwegians only ($r_s = 0.47$, $p = 0.04$). In conclusion, despite similar adipose tissue distribution in the two groups Pakistanis were more insulin resistant, with a negative correlation of VAT to insulin sensitivity, not present in Norwegians. The correlation of adipose tissue to Leptin, CRP and IL-1RA showed ethnic differences.

Key Words: *Anthropometry, C-reactive protein, tomography, X-ray computed, immigrants, insulin resistance, Interleukin 1 receptor antagonist, human, Leptin, Southern Asia, subcutaneous adipose tissue, visceral adipose tissue*

Introduction

Immigrants from South Asia to Western countries show increased insulin resistance [1] and a high prevalence of type 2 diabetes mellitus (T2DM) [2,3]. The association between insulin resistance and obesity is well established. However, several reports indicate that commonly used methods of anthropometric measurements, like the body mass index (BMI), may be inadequate as indicators of metabolic risk in South Asians, who seem to develop insulin resistance and T2DM at levels of BMI that are considered low risk in the Western population [4,5].

Body composition can be measured using dual X-ray absorptiometry (DXA) or bioelectrical impedance analysis (BIA). However, these methods discriminate poorly between the various abdominal adipose tissue compartments such as subcutaneous (SAT) and visceral adipose tissue (VAT). Using computed tomography (CT) or magnetic resonance imaging (MRI), the adipose tissue compartments can be measured more accurately [6]. Previous studies have indicated that abdominal SAT and VAT are differently related to metabolic risk [7], and that VAT, and possibly deep subcutaneous adipose tissue (DSAT), is closely related to insulin resistance. DSAT has been shown by several authors to be functionally and morphologically different from superficial subcutaneous adipose tissue (SSAT)

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[8,9]. Increased fatty infiltration in the liver is also associated with overall insulin resistance [10].

Obesity and insulin resistance are accompanied by low-grade systemic inflammation with increased plasma levels of inflammatory markers, in part released from adipose tissue. Recent studies have suggested that the various adipose tissue compartments may differ in their inflammatory potential [11–13] in addition to their differing relation to insulin resistance.

The aim of this study was to investigate: (i) Possible differences in the abdominal adipose tissue distribution between T2DM subjects from Norway and Pakistan; (ii) whether ethnic differences in insulin resistance could be explained by differences in adipose tissue distribution; and (iii) whether possible ethnic differences in insulin resistance and body composition could be related to plasma levels of inflammatory markers.

Materials and methods

Ethics

The study was approved by the Eastern Norway Regional Committee for Medical and Health Research Ethics, and conformed to the Helsinki declaration. Informed written consent was obtained from each participant prior to any study related procedure.

Patients

The study population has previously been described [14]. Briefly, 18 Pakistani and 21 Norwegian patients (49% men), aged 29–45 years, with confirmed T2DM, were recruited from two hospital out-patient clinics, at Lovisenberg Deaconess Hospital and Aker University Hospital in Oslo. Clinical characteristics of the two ethnic groups are shown in Table I. The Pakistanis had longer duration of diabetes and higher HbA1c than the Norwegians. Results have therefore been adjusted for HbA1c and diabetes duration through multiple regression analyses.

Anthropometrical measurements

Height and weight were measured with participants wearing light clothing and no shoes. BMI was calculated as weight in kg/(height in m)^2. Waist and hip circumferences were assessed with a tape measure with spring scale to ensure equal traction at every measurement, measuring at mid-point between the lowest rib margin and the iliac crest, and at the level of the major trochanter, respectively. BIA was performed on 17 of the Norwegian and 14 of the Pakistani patients, on a Tanita Body Composition Analyzer BC-418 (Tokyo, Japan), providing measurements of percentage total body fat (%TBF), body fat mass in kilograms (BFM) and fat free mass in kilograms (FFM). All subjects were fasting and had voided urine before measurements.

Computed tomography measurements

CT was performed using a CT Somatom (Erlangen, Germany) with the patient examined in a supine position, arms extended above the head. Three single axial scans were performed without intra-venous contrast medium, through (i) the liver and the spleen, at the level of Th12, (ii) the mid-abdomen, 10 cm above L4/L5 in men and 5 cm above L4/L5 in women (Figure 1A) [15], and (iii) the thighs, at mid-distance between the anterior-superior iliac spine and the upper margin of the patella. CT parameters were 120 kV, 100 mAs, and slice thickness 4 mm. The dicom images were analysed using Osirix v 3.2.4, 32 bit.

Table I. Clinical characteristics of T2DM patients according to ethnic group.

	Norwegians $n = 21$	Pakistanis $n = 18$	p
Males n (%)	10 (48%)	9 (50%)	
Age (years)*	42 [15]	41 [16]	0.865
Years with diabetes*	5 [16]	9 [17]	**0.021**
Diabetes treatment n (%):			
Lifestyle ± OAD / Insulin ± OAD	11 (52%)/10 (48%)	5 (28%)/13 (72%)	0.119
Weight (kg)	106.8 [49.3]	90.1 [86.9]	**0.009**
BMI (kg/m^2)*	37.2 [15.0]	30.9 [35.2]	**0.008**
Waist circumference (cm)	114.3 [50.0]	106.5 [59.5]	0.102
Waist-hip ratio	1.00 [0.34]	1.01 [0.36]	0.575
Total body fat (%)	36.9 [29.9]	34.2 [27.4]	0.395
Body fat mass (kg)	39.5 [43.7]	28.0 [25.5]	**0.005**
Lean body mass (kg)	67.1 [39.4]	54.2 [34.2]	**0.007**
HbA1c (%)*	7.3 [5.6]	8.7 [6.5]	**0.022**

Data are shown as mean [range] with comparisons using Student's t-test. *For parameters with non-normal distribution median [range] are given, with comparisons using the Mann-Whitney U test. Diabetes treatment is compared using Chi-square test for independence. OAD: oral anti-diabetic treatment. $p < 0.05$ in bold type.

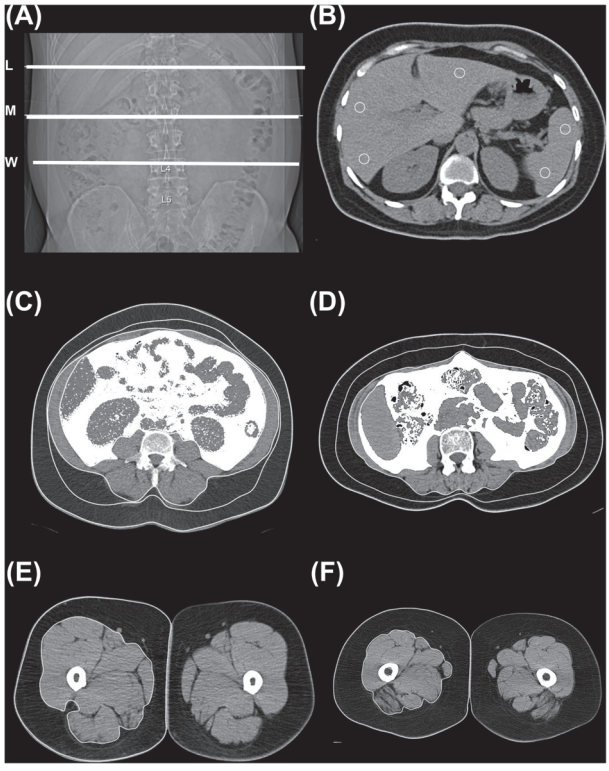

Figure 1. Computed tomography measurements in Norwegian and Pakistani patients with type 2 diabetes mellitus (T2DM). (A) Abdominal scanogram marked with the levels of the two axial CT scans, with the most cranial through the liver and spleen (L), and the caudal scan 10 cm above L4/L5 in men (M) or 5 cm above L4/L5 in women (W). (B) Axial CT scan showing three regions of interest (ROI) in the liver and two in the spleen, for measurement of attenuation in Hounsfield Units. (C) and (D) Axial mid-abdominal CT-scans showing tracking of fat and muscle compartments and highlighting of VAT in a Norwegian woman (C) and Pakistani woman (D) with large and small abdominal muscle areas respectively. (E) and (F) CT-scans through thighs in a Norwegian (E) and a Pakistani (F) woman showing the tracking of fat and muscle compartments.

The fat content of the liver was measured based on attenuation values in Hounsfield Units (HU). The liver-spleen ratio (LS-ratio) was calculated based on the mean of three measurements in the liver (2 right lobe and 1 left lobe) and two measurements in the spleen with a region of interest (ROI) 80 mm^2 (Figure 1B). In mid-abdomen, the circumferences for SAT were tracked for both SSAT and DSAT compartments, divided by the superficial fascia [16]. The abdominal muscle compartment area was traced by the inner limit of DSAT and the outer limit of VAT. The VAT compartment was measured by tracking the inner abdominal circumference, and measuring pixels with fat highlighted between -30 and -190 HU (Figure 1C and D). The right thigh was selected for measuring thigh SAT and muscle compartments, tracing around the fat and muscle compartments as shown (Figure 1E and F).

Euglycemic clamp

The euglycemic clamp method has previously been described [14]. Briefly, the patients underwent a two-step euglycemic, hyperinsulinemic clamp, using a modified version of the method originally described by De Fronzo et al. [17]. Human insulin (Actrapid®, Novo Nordisk, Copenhagen, Denmark) 300 mU/mL and glucose 200 mg/mL were infused through a teflon catheter in a vein at the left elbow of the patient. Insulin was diluted in NaCl 0.9%, after having first added 2 mL of the patient's own blood, to avoid insulin sticking to the walls of the bag. All blood samples were drawn from a Teflon catheter in a vein at the right elbow, kept open by a slow infusion of NaCl 0.9%. The right arm was kept at 37°C by a heating sleeve connected to a thermal control unit (Swetron AB, Veddestad, Sweden), to arterialize blood samples. Two successive clamp steps of 40 and 400 mU/m^2/min of insulin were performed, with a minimum duration of 100 min, and at least 30 min of stable euglycemia for each clamp step. The body surface area was calculated using Mostellers equation [18]. Plasma glucose was measured every five min using a OneTouch Ultra glucose measuring device (LifeScan, Milpitas, CA, USA), with control measurements every 30 min by the glucose oxidase method, on a Glucose Analyzer II (Beckman Instruments, Fullerton, CA, USA). The glucose infusion rate (GIR) in µmol/m^2/min was calculated for both clamp steps. The Insulin Sensitivity Index (ISI) was expressed as the ratio of the GIR to the prevailing mean serum insulin levels at the end of each clamp step ([GIR/I] $\times 100$). Data presented here are from the first step of the clamp. Two Norwegian and two Pakistani patients did not attain euglycemia during the first step of the clamp, and were excluded from this part of the clamp analyses. After excluding these patients, there were 46% men in the first step of the clamp.

Blood samples

Fasting plasma glucose was measured by the glucose oxidase method on a Glucose Analyzer II (Beckman Instruments). Serum insulin was analyzed using the radioimmunoassay (RIA) kit, formerly from Linco Research, presently available from Millipore Corp. (Billerica, MA, USA). Plasma levels of adiponectin and leptin were analyzed using RIA kits from Millipore Corp. (Billerica, MA, USA), (also formerly from Linco Research). Plasma high sensitive C-reactive protein (CRP) was measured using a DuoSet ELISA kit from R&D Systems (Minneapolis, MN, USA). Plasma interleukin-1 receptor antagonist (IL-1RA) was measured using CytoSet from Invitrogen Corporation (Carlsbad, CA, USA), with streptavidin-horseradish peroxydase from R&D Systems. Plasma measurement of interleukin-6 (IL-6)

was performed using a High Sensitivity ELISA kit from Abcam plc. (Cambridge, UK).

Statistical analyses

Data are presented as mean ± SD or median [interquartile range] unless otherwise specified. We analyzed non-normally distributed data using non-parametric methods, or log-transformed, as appropriate. Student's *t*-tests or Mann-Whitney U tests were used for comparison of continuous variables between groups. For comparison of categorical data between patient groups, the Chi-square test for independence was used. Spearman's correlation coefficient (r_s) was used. Multiple linear regression analyses were performed, with log-transformation of parameters when needed, to ensure no violation of the assumptions of normality, linearity and homoscedasticity. A two-sided p-value < 0.05 was regarded as significant, but owing to the large number of comparisons, particular attention should be directed towards analyses where p-values are < 0.01. Statistical analyses were performed with SPSS 19.0 for windows (SPSS Inc., Chicago, IL, USA).

Results

Adipose tissue and muscle distribution

The Norwegians had higher weight, BMI, BFM and FFM than the Pakistanis, but there was no significant difference in the %TBF. (Table I). The higher HbA1c and longer diabetes duration in the Pakistani group did not have any significant impact on the results presented.

Liver fat infiltration and abdominal and thigh adipose tissue compartments did not differ between the two ethnic groups (Table II). The relative size of VAT as determined by the VAT/SAT ratio, was significantly smaller in women than in men (0.49 [0.35] vs. 1.26 [0.84], $p < 0.001$), but again, there was no ethnic difference. For women, abdominal and thigh muscle compartments were significantly larger in the Norwegian group, while for men, there was only a tendency towards larger abdominal muscle compartment in the Norwegians (Table II).

SAT was found to correlate well to several anthropometrical markers of obesity, such as BMI, waist circumference and %TBF in both groups. VAT on the other hand, correlated less well to BMI and waist circumference, with particularly weak correlations in the Norwegian group. Moreover, VAT was paradoxically, negatively correlated to %TBF in the Norwegian group with no significant correlation in the Pakistani group (Table III).

Insulin sensitivity

Insulin sensitivity (ISI_{40}) was negatively correlated to VAT and positively correlated to liver attenuation and LS-ratio, but not to SAT, in the total patient group. However, when analyzing each ethnic group separately, the negative association with VAT and liver fat was present in the Pakistani group while it was not significant in the Norwegian group (Table IV). Using multiple regression analysis examining predictors of insulin sensitivity, we found that 38.6% of $LogISI_{40}$ variation was explained by the combination of VAT ($p < 0.001$), sex ($p = 0.015$) and ethnicity ($p = 0.046$), (model significance: $p = 0.002$). The total DSAT + VAT compartment was also significantly correlated to ISI_{40}, and again, this did reach statistical significance in the Pakistani but not in the Norwegian group (Table IV). In line with this, the correlation between DSAT + VAT and ISI_{40} became stronger ($p = 0.001$) when adjusting for ethnicity ($p = 0.023$) in a multiple regression analysis with $logISI_{40}$ as dependent variable (model significance: $p = 0.003$, $R^2 = 0.31$).

Table II. Adipose tissue and muscle distribution in Norwegians and Pakistanis with T2DM.

	Women			Men		
	Norwegian $n = 11$	Pakistani $n = 9$	p	Norwegian $n = 10$	Pakistani $n = 9$	p
SSAT (cm^2)[a]*	270 [86]	237 [160]	0.36	155 [75]	125 [117]	0.18
DSAT (cm^2)[a]*	161 [122]	125 [89]	0.59	105 [125]	74 [39]	0.09
VAT (cm^2)[b]*	217 ± 71	210 ± 59	0.84	282 ± 49	264 ± 73	0.53
VAT/SAT ratio[b]*	0.52 ± 0.20	0.57 ± 0.23	0.59	1.21 ± 0.74	1.56 ± 0.94	0.39
SAT thigh (cm^2)[a]	192 [90]	169 [73]	0.27	105 [52]	95 [71]	0.29
Liver attenuation (HU)[b]	44.9 ± 23.0	50.8 ± 11.5	0.49	52.7 ± 9.0	47.8 ± 12.4	0.33
LS ratio[b]	0.88 ± 0.45	0.98 ± 0.21	0.57	0.98 ± 0.17	0.88 ± 0.22	0.31
Abdominal muscle area (cm^2)[b]*	188 ± 28	157 ± 21	**0.02**	220 ± 12	197 ± 31	0.07
Thigh muscle area (cm^2)[b]	156 ± 25	130 ± 21	**0.03**	194 ± 19	182 ± 38	0.39

[a]Data are shown as median [IQR], two-sided p from Mann-Whitney U test. [b]Data are shown as mean ± SD, two-sided p from Student's t-test. SSAT, superficial subcutaneous adipose tissue; DSAT, deep subcutaneous adipose tissue; VAT, visceral adipose tissue; SAT thigh, subcutaneous adipose tissue in right thigh; Liver attenuation in Hounsfield units (HU); LS ratio, liver-spleen ratio. p-values < 0.05 are in bold type.
*Pakistani women: $n = 8$.

Table III. Correlations of adipose tissue compartments to anthropometrical measurements.

	SAT		VAT	
	Nor	Pak	Nor	Pak
BMI				
r_s	**0.87**	**0.74**	−0.02	0.46
p	**<0.001**	**0.001**	0.94	0.06
Waist circumference				
r_s	**0.59**	**0.72**	0.23	0.45
p	**0.006**	**0.001**	0.34	0.07
% TBF				
r_s	**0.72**	**0.92**	**−0.57**	0.05
p	**0.001**	**<0.001**	**0.017**	0.88

Nor, Norwegians; Pak, Pakistanis; r_s, Spearman's correlation coefficients; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue; BMI, body mass index; %TBF, percentage total body fat. p-values < 0.05 are in bold type.

Adipokines and inflammatory markers

Plasma levels of leptin, CRP and IL-1RA showed significant correlations with adipose tissue compartments, while no association was shown for adiponectin or IL-6 (Table V). Plasma levels of leptin were highly positively correlated to abdominal and thigh SAT in both groups, with the strongest correlations in the Pakistani group. In fact, in a multiple regression analysis exploring predictors of plasma leptin, we found that 77.8% of the variation in leptin was explained by SSAT ($p < 0.001$), sex ($p < 0.001$) and ethnicity ($p = 0.046$) (model significance: $p < 0.001$).

Table IV. Correlations of adipose tissue compartments to the Insulin Sensitivity Index in Norwegian and Pakistani patients with T2DM.

	ISI_{40}		
	Tot $n = 34$	Nor $n = 19$	Pak $n = 15$
SSAT			
r_s	−0.253	−0.308	−0.339
p	0.150	0.199	0.216
DSAT			
r_s	−0.068	−0.160	−0.141
p	0.702	0.514	0.615
VAT			
r_s	**−0.424**	−0.249	**−0.704**
p	**0.013**	0.304	**0.003**
DSAT+VAT			
r_s	**−0.432**	−0.338	**−0.781**
	0.011	0.157	**0.001**
Liver Att.*			
r_s	**0.404**	0.319	0.427
p	**0.016**	0.182	0.099
LS-ratio*			
r_s	**0.401**	0.329	0.389
p	**0.017**	0.169	0.137

Tot, total; Nor, Norwegians; Pak, Pakistanis; r_s, Spearman's correlation coefficients; SSAT, superficial subcutaneous adipose tissue; DSAT, deep subcutaneous adipose tissue; VAT, visceral adipose tissue; Liver Att., Liver attenuation. Correlation coefficients with p-values < 0.05 are in bold type. *Total $n = 35$, Pakistani $n = 16$.

Moreover, CRP was positively correlated to abdominal SAT in the Pakistani group, and IL-1RA to abdominal and thigh SAT in the Norwegian group only.

Discussion

In this study, we report both ethnic similarities and differences in the distribution of adipose and muscle tissue, as well as their relations to insulin sensitivity and inflammatory markers.

The Norwegian and Pakistani groups showed no significant differences in the areas of adipose tissue compartments measured by CT in abdomen or thigh, although the Norwegian subjects were overall significantly larger, with higher total BFM, FFM and BMI. However, we observed ethnic differences in how abdominal adipose tissue compartments such as SSAT, DSAT and VAT related to insulin sensitivity and inflammatory markers. VAT appeared to be more metabolically active in the Pakistani than in the Norwegian group, displaying strong negative correlations to insulin sensitivity. In addition, an association between CRP and abdominal SAT was only seen in the Pakistani group, suggesting a stronger link between SAT and inflammation in Pakistanis with T2DM as compared with the Norwegians.

The significant positive correlations of CRP to SAT compartments, but not to VAT in our Pakistani patients are at variance with some previous reports [12,13]. This may be due to the higher BMI, %TBF and HbA1c values in both our ethnic groups than those reported in other studies, making comparisons difficult. In a cohort of severely obese subjects, CRP was found to be a non-specific marker of obesity, and not related to specific adipose tissue compartments or metabolic dysregulation like the metabolic syndrome, T2DM or non-alcoholic steato-hepatitis [19]. SAT in our study correlated better than VAT to general measurements of obesity, thus possibly explaining these findings in our Pakistani group. Nonetheless, CRP is a reliable down-stream marker of inflammation, and the positive correlation of CRP to SAT in the Pakistani group suggests a more inflammatory potential in SAT in the Pakistani group.

We found similar abdominal adipose tissue distribution in the Pakistani and Norwegian groups. Recently, Lear and co-workers demonstrated that healthy South Asian men and women had higher levels of both SAT and VAT than a similar group of subjects of European ancestry [20]. The same authors have however previously shown that the ethnic differences in VAT between the South Asians and Europeans in this cohort were only evident when waist circumference was lower than 105 cm [21]. Other authors have found no ethnic differences in adipose tissue distribution, at least in men [22,23]. In our study population the mean waist circumferences were wider than 105 cm in both ethnic groups, and

Table V. Correlations of adipose and muscular tissue compartments to markers of inflammation.

	Adiponectin		Leptin		CRP		IL-6		IL-1RA	
	Nor ($n=21$)	Pak ($n=16$)	Nor ($n=21$)	Pak ($n=16$)	Nor ($n=20$)	Pak ($n=16$)	Nor ($n=20$)	Pak ($n=16$)	Nor ($n=20$)	Pak ($n=16$)
SSAT										
r_s	−0.18	0.27	**0.76**	**0.86**	−0.28	0.50	0.07	0.30	**0.47**	0.25
p	0.44	0.29	**<0.001**	**<0.001**	0.23	0.05	0.76	0.26	**0.04**	0.35
DSAT										
r_s	0.04	−0.10	0.44	**0.68**	0.00	**0.56**	−0.23	0.25	−0.35	0.26
p	0.85	0.69	0.05	**0.003**	0.99	**0.03**	0.33	0.35	0.13	0.33
SAT										
r_s	−0.11	0.19	**0.67**	**0.88**	−0.18	**0.55**	−0.08	0.37	0.43	0.24
p	0.63	0.49	**0.001**	**<0.001**	0.44	**0.03**	0.73	0.16	0.06	0.37
VAT										
r_s	−0.38	−0.06	−0.12	−0.03	0.10	−0.212	0.15	−0.33	0.15	0.08
p	0.09	0.82	0.60	0.90	0.68	0.43	0.52	0.22	0.53	0.76
VAT/SAT										
r_s	−0.11	−0.21	**−0.60**	**−0.77**	0.02	**−0.60**	0.05	−0.45	−0.29	−0.16
p	0.63	0.42	**0.004**	**<0.001**	0.94	**0.01**	0.85	0.08	0.21	0.56
SAT thigh*										
r_s	0.04	0.23	**0.56**	**0.88**	−0.08	0.24	0.21	−0.01	**0.49**	0.15
p	0.88	0.37	**0.01**	**<0.001**	0.74	0.36	0.38	0.96	**0.03**	0.56
Liver att.*										
r_s	−0.11	0.38	−0.25	0.16	0.30	0.20	0.23	0.45	−0.38	−0.35
p	0.65	0.12	0.28	0.52	0.20	0.44	0.32	0.07	0.10	0.17
Abdo musc.										
r_s	−0.01	−0.09	−0.39	−0.23	−0.06	−0.01	−0.14	−0.10	−0.28	0.44
p	0.95	0.73	0.08	0.38	0.81	0.98	0.56	0.71	0.24	0.09
Thigh musc.*										
r_s	0.17	−0.40	−0.43	−0.33	0.13	−0.11	−0.31	−0.24	−0.26	0.39
p	0.46	0.11	0.05	0.18	0.59	0.67	0.18	0.35	0.26	0.12

CRP, C-reactive protein; IL-1RA, Interleukin-1 receptor antagonist; IL-6, interleukin-6; r_s, Spearman's correlation coefficients; SSAT, superficial subcutaneous adipose tissue; DSAT, deep subcutaneous adipose tissue; SAT, total subcutaneous adipose tissue; VAT, visceral adipose tissue; VAT/SAT, visceral-to-subcutaneous adipose tissue ratio; SAT thigh; subcutaneous adipose tissue in thigh; Liver att., liver attenuation; Abdo musc., abdominal musculature; Thigh musc., thigh musculature and various adipokines and markers of inflammation. Correlation coefficients with p-values <0.05 in bold type. *For Pakistani patients $n=17$.

BMI was higher than in the studies mentioned (Table I). The higher degree of adiposity in our study, where the subjects also had established T2DM, could therefore have attenuated ethnic differences in VAT, and possibly also in SAT.

Leptin levels were strongly and positively correlated to areas of SAT in both ethnic groups, as expected. In our patients, IL-1RA, like leptin, correlated positively to SAT in abdomen and thigh in the Norwegian, but not the Pakistani group. There was no significant correlation of IL-1RA to VAT. Our results may indicate that IL-1RA is a more important factor in the Norwegian than in the Pakistani group. However, due to relatively small numbers included, conclusions should be drawn with care.

In our Norwegian group, the adipose tissue compartments seemed to be less important for determining insulin sensitivity. The mean age at diagnosis of T2DM in subjects from South Asia living in Norway has previously been found to be more than 10 years lower than in ethnic Norwegians [24]. We might speculate that by having selected young Norwegian T2DM subjects these could have a stronger genetic predisposition to diabetes. Obesity might therefore be of somewhat less importance for the development of T2DM in the Norwegian group. The Pakistani subjects on the other hand, were closer in age to the average Pakistani T2DM patient in Norway. The negative correlation between insulin sensitivity and VAT, seen only in the Pakistani group, may also reflect genuine ethnic differences in the composition and metabolic character of VAT, with VAT being more metabolically active in Pakistanis than in Norwegians.

The inflammatory markers we chose to study were the major markers that have been shown in other studies to be linked to obesity and type 2 diabetes. Adiponectin and leptin are known as adipokines, since they are produced mainly in adipose tissue. CRP is an acute phase reactant known to be elevated in low-grade, metabolic inflammation, stimulated by IL-6. Leptin has been shown to induce monocyte expression of IL-1RA [25], and increased levels of IL-1RA are correlated to the hyperleptinemia of obesity [26]. IL-1RA has been shown to correlate to SAT and especially to VAT [27]. Increasing IL-1RA levels have also been associated with the development of T2DM [28]. We did not analyze

TNFα since it is an inflammation marker that possibly acts primarily in a paracrine manner, and hence is difficult to measure in the circulation.

The strengths of this study include employing the two-step euglycemic, hyperinsulinemic clamp for measuring insulin sensitivity, and having both CT and BIA measurements of body composition. There are differences in both HbA1c and diabetes duration between the two groups. These differences are also seen in other studies [24,29,30], and reflect differences that are found in the general population with type 2 diabetes. Thus, an attempt to match for these parameters could in fact have created a selection bias. We have taken care to adjust for HbA1c and diabetes duration, and found that they did not influence on the results. We have also previously shown that the ethnic differences in insulin sensitivity between these groups could not be explained by the differences in HbA1c or diabetes duration [14]. The groups we have studied are of restricted numbers, and this constitutes a limitation, increasing the risk of not being able to detect small but possibly important differences. Furthermore, we report many statistical tests without correction for multiple testing, with the risk of reporting p-values < 0.05 by chance. This is an exploratory study, and we therefore deemed it appropriate to show uncorrected p-values [31]. We have instead focused on the most robust statistical findings in our discussion and conclusions, and have taken care to give the exact p-values throughout the manuscript, to facilitate the possibility for the reader to judge. By recruiting patients from hospital out-patient clinics only, there may be a selection bias towards including subjects with T2DM that is not easily treated in general practice, and the findings should be interpreted in this context. Lastly, we have no information about degree of physical activity, where a difference between the groups could influence the results. We have only asked the participants to refrain from hard physical exercise during the two days prior to the euglycemic clamp.

In conclusion, we report that the Norwegian and Pakistani group differ in body composition, but are still similar in abdominal and thigh adipose tissue distribution. In spite of similar adipose tissue distribution there is evidence of ethnic differences in the importance of adipose tissue distribution for insulin sensitivity, where visceral adipose tissue seems to be especially important in the metabolic dysregulation in Pakistanis. We have also found differences in the relationship between adipose tissue distribution and some adipokines and markers of inflammation, although further investigation in this field is needed.

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