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Maastricht University

Faculteit Geneeskunde en Levenswetenschappen
School voor Levenswetenschappen
master in de biomedische wetenschappen

Masterthesis

Association of (pre)diabetes and pancreatic fat

Yasmine Amasaad

Scriptie ingediend tot het behalen van de graad van master in de biomedische wetenschappen, afstudeerrichting klinische biomedische wetenschappen

PROMOTOR :

Mevrouw Carla VAN DER KALLEN

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Association of (pre)diabetes and pancreatic fat.

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Association of (pre)diabetes and pancreatic fat.

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Keywords: Pancreatic fat, prediabetes, type 2 diabetes, fat depots, in- and out- phase MRI images, head, body, tail

ABSTRACT

Background- Type 2 diabetes mellitus (T2DM) is caused by the combination of impaired insulin production and insulin resistance. The dysfunction in insulin release by the pancreas might (partially) be caused by pancreatic fat accumulation. The aim of this study is to determine the association between (pre)diabetes and pancreatic fat.

Methods- Cross-sectional study of the Maastricht study data. A population of 900 individuals were randomly selected by random selection of 300 individuals (150 men and 150 women) with NGM (normal glucose metabolism), 300 with prediabetes and 300 with T2DM. The fasting glucose levels and information about diabetes medication were used to determine glucose metabolism status. Glucose metabolism status was defined according to the WHO 2006 criteria. Pancreatic fat is quantified by in- and out- phase MRI images using ImageJ. Multiple regression analysis was performed to address the associations between the percentage of pancreatic fat and (pre)diabetes.

Results- Individuals with T2DM have the highest average pancreatic fat percentage (9.04 % ±3.57 %), compared with prediabetes (8.07 % ±3.60 %) and NGM (6.79 % ±3.55 %). Moreover, (pre)diabetes is associated with pancreatic fat after adjusting for age and sex. Further adjustment for lifestyle factor had no large effect on the association. Visceral fat, but not BMI and liver fat, attenuated the association of T2DM with pancreatic fat.

Conclusion – (pre)diabetes is associated with pancreatic fat. These findings suggest that pancreatic fat could be a promising biomarker and/or part of the pathophysiology of the development of T2DM.

SAMENVATTING

Type 2-diabetes (T2DM) wordt veroorzaakt door de combinatie van verminderde insuline productie van de βcellen en insuline resistentie. De onvoldoende insuline productie door de pancreas zou kunnen veroorzaakt worden door de vetstapeling op het orgaan. Het doel van deze studie is om te achterhalen als er een associatie is tussen (pre)diabetes en pancreas vet. Hierdoor ontstond een cross-sectionele met de data van de

Maastricht studie. In totaal waren het 900 deelnemers, die random geselecteerd werden uit de Maastricht studie data, met 300 individuen (150 man, 150 vrouw) met een normaal glucosemetabolisme, 300 prediabeten en 300 met T2DM. De nuchtere glucosewaarde werd verzameld om de diabetes status te bepalen. De pancreas vet werd gekwantificeerd door in-en-out phase MRI-beelden, met het gebruik van ImageJ. Zo vonden we dat de T2DM (9.04 % ±3.57 %), het hoogste pancreas vet had, in vergelijking met de prediabeten (8.07 % ±3.60 %) en NGM'ers (6.79 % ±3.55 %). Meer zelfs, (pre)diabeten waren geassocieerd met pancreas vet, na correctie voor leeftijd en geslacht. Ook had levensstijl geen effect op de associatie tussen (pre)diabetes en pancreas vet. Het resultaat was dat er een associatie is tussen T2DM en pancreas vet, onafhankelijk was van body mass index, levervet en subcutaan vet, maar niet van visceraal vet. Daardoor kunnen we besluiten dat er een associatie is tussen (pre)diabetes en pancreas vet. Deze resultaten suggereren dat pancreas vet een biomarker kan zijn en/of deel uitmaakt in de pathofysiologie van het ontwikkelen van T2DM.

INTRODUCTION

Type 2 diabetes - One of the fastest-growing health challenges of the 21st century is diabetes mellitus. Over the past 20 years, the number of adults living with diabetes has more than tripled. This disease has two common forms: type 1 diabetes mellitus (T1DM) and type 2 diabetes mellitus (T2DM), with the former having a prevalence of 10% and the latter accounting for approximately 80% (1). T1DM is a chronic autoimmune disorder characterized by insulin deficiency, where insulin production is disrupted. T1DM is most commonly seen in children or young adults, but it can manifest at any age (2). The cause of the disease is still unknown (2). T2DM is a progressive disease characterized by chronic hyperglycaemia. It is caused by the combination of impaired insulin production and insulin resistance. The hormone insulin is secreted by the pancreas and ensures glucose uptake in insulin-sensitive cells (3). When insulin resistance occurs, cells cannot respond fully to insulin, and the hormone becomes less effective. The circulating insulin level must increase to induce glucose lowering. Increased β-cell activity in the pancreas to cope with demand causes it to be exhausted over time.

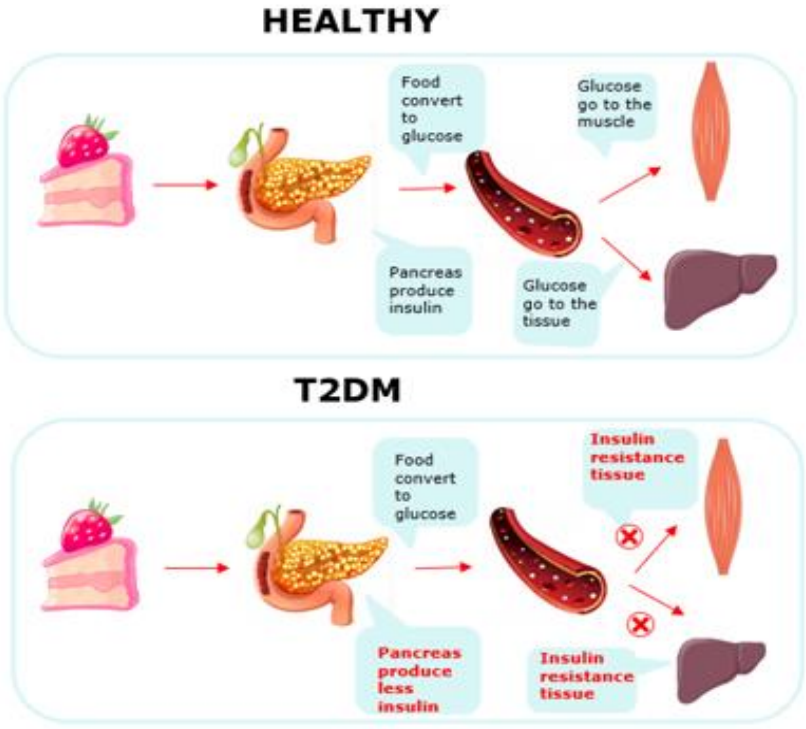

Fig. 1 –Different metabolism in healthy individuals and type 2 diabetes. *Patients with T2DM produce less insulin and are insulin resistance. This results in more hyperglycaemia (created with biorender).*

This is the major mechanism for the development of T2DM (figure 1) (4). It is usually diagnosed in older adults but is increasingly seen in children and younger adults (1).

Before the development of T2DM, patients can be diagnosed with prediabetes, which is an intermediate form of hyperglycaemia exhibited by glycaemic parameters above normal but below diabetes thresholds (5). Prediabetes is known to start insulin resistance and impair insulin production (5). The development of prediabetes and T2DM is a multifactorial factor. Lifestyle, including obesity, physical inactivity, poor nutrition, and inappropriate diet, is one factor (6). Genetic factors and family history are also risk factors for T2DM (7,8). If blood glucose levels are not consistently regulated in individuals diagnosed with T2DM, hyperglycaemia can manifest a number of long-term complications, such as cardiovascular disease, kidney failure, retinopathy, and neuropathy. These complications can be observed in individuals with prediabetes (4,9,10). Moreover, these complications affect patients' quality of life. T2DM patients lose quality life years and die earlier than individuals without T2DM (1) (4).

Obesity is an abnormal or extensive fat adiposity accumulation that can impair the health of an individual (11). Obesity consists of increased subcutaneous fat deposition. Subcutaneous fat is the fat underneath the skin, which can lead to visceral adiposity and increased ectopic fat deposition in the liver, skeletal muscle, heart, and pancreas (10,12). Interestingly, visceral fat and ectopic fat are closely related to metabolic dysfunction, such as insulin resistance, hypertension, and cardiovascular disease (13).

Pancreatic fat - As described earlier, obesity can increase ectopic fat deposition around the pancreas (14). This condition is called pancreatic fat, and it is characterized by ectopic fat deposition. Fatty pancreas develops mainly through obesity or metabolic syndrome (13,14). Other etiological factors are congenital syndromes, toxic substances, or viral diseases (15). Fat accumulation in pancreas cells causes inflammation, which leads to endocrine or exocrine dysfunction in the pancreas (9). The toxic effect on the pancreatic acinar and β-cells affects insulin secretion and causes chronic pancreatitis, pancreatic cancer, and acute pancreatitis (16). Predictors of pancreatic fat are aging and sex. Aging leads to fat redistribution from subcutaneous to abdominal depots in the liver, muscles, pancreas, and other ectopic sites (9). Aging plays a role in the development of pancreatic fat (17). Another predictor of pancreatic fat is the significant difference in body fat distribution between males and females. Women tend to have a pear-shaped body, making them more prone to subcutaneous fat development. Conversely, men are more prone to visceral fat development, as they often have apple-shaped figures. Men therefore have more visceral fat, which leads to pancreatic fat (18).

Aim of the study- T2DM results from a combination of varying degrees of insulin secretion defects. This dysfunction in insulin release may be caused by pancreatic fat and can induce the development of prediabetes and T2DM. If pancreatic fat reflects (or causes) β-cell dysfunction, this can be a possible hallmark for the detection of (pre)diabetes at an early stage. Knowledge regarding the pathophysiology of fatty pancreas is currently limited (9). Only a few studies have been performed on T2DM as associated with pancreatic fat (16,19-21). Clinical consequences of fatty pancreas also remain unclear; therefore, **the ultimate goal of this report is to determine the association between pancreatic fat percentage and (pre)diabetes**. We quantify fat in the pancreas head, body, and tail to elucidate the relationship between pancreatic fat and (pre)diabetes and determine the association between adiposity and pancreatic fat percentage. We hypothesize that there is an association between pancreatic fat percentage and (pre)diabetes and between adiposity and pancreatic fat percentage.

EXPERIMENTAL PROCEDURES

Study design and participants – The Maastricht Study, an observational, prospective, population-based cohort study. The focus of the study is on the etiology, pathophysiology, complications, and comorbidities of T2DM and is characterized by an extensive phenotyping approach (22). The only inclusion criteria are the individuals aged between 40 and 75 years and living in the southern part of the Netherlands were eligible for participation. Participants were recruited through mass media campaigns and via mailings from the municipal registries and the regional Diabetes Patient Registry (22).

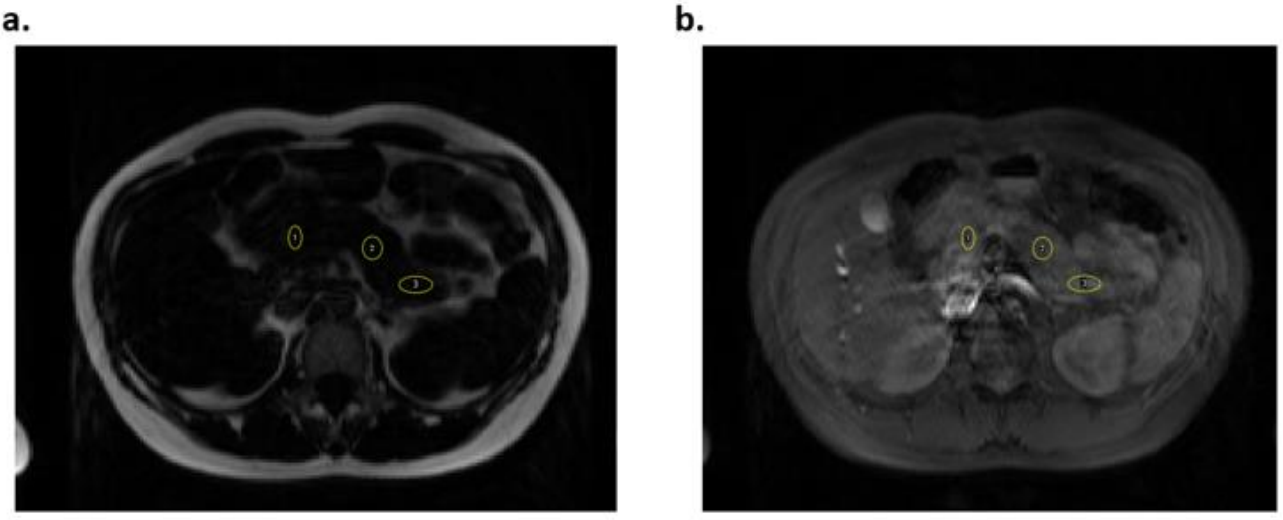

Fig. 2 – **Images of pancreas of the individuals.** *In and out phase images of the pancreas to evaluate the pancreatic fat. Determined by double-echo chemical shift gradient gradient-echo MR imaging. The yellow circles show the region of interest: 1 = head, 2: body and 3 = tail. Figure 1a present the fat image (out phase images) and figure 1b present the water image (in phase images).*

The research described in this report was a prospective cross-sectional study design, with the data from the Maastricht Study, while focusing on the association between (pre)diabetes and pancreatic fat percentage. MRI measurements of the pancreas were implemented from December 2013 onwards an available in 5180 participants. This study focused on a group of 900, choosing by randomization. The groups consist of 300 individuals (150 men and 150 women) with NGM (normal glucose metabolism), 300 with prediabetes and 300 with T2DM.

Assessment diabetes status – To determine the glucose metabolism status of the individuals, fasting glucose level was measured in venous blood samples, and information about diabetes medication were used to determine glucose metabolism status. Glucose metabolism status was defined according to the WHO 2006 criteria into NGM, prediabetes [impaired fasting glucose, impaired glucose tolerance, and T2DM (23). Individuals without T1DM on diabetes medication were classified as having T2DM (22).

Assessments Pancreatic fat - Pancreas content was assessed through Dixon MR imaging using a 3.0 T MRI system (MAGNETOM Prismafit, Siemens Healthineers, Erlangen, Germany) with body matrix and supine radiofrequency coils. After a scout scan, transversal two-dimensional T2-weighted True Fast Imaging with Steady-State Free Precession (T2w TRUFI) images were acquired through the pancreas with the following parameters: voxel size: 1.2×1.2×5.0 mm 3, repetition time (TR); 422 ms, echo time (TE): 1.65 ms, flip angle: 60, number of signal averages: 1, parallel imaging (GRAPPA) factor: 2. Next, transversal two-dimensional turbo spin echo Dixon MR images were acquired through the pancreas during a breathhold using the following parameters: voxel size: 2.0×2.0×6.0 mm, number of slices: 4, TR: 500 ms, TE: 31 ms, turbo factor: 5, number of signal averages: 1, parallel imaging (GRAPPA) factor. We used the ImageJ Polygon tool to select a region of interest (ROI) in the parenchymal tissue of the pancreas head, body, and tail (figure 2). For the selection of the ROI, the oval tool of image J was used. We intend to draw the ROI, on one of the Dixon MR images, as large as possible yet not close to the borders to exclude other parts, such as blood vessels or visceral fat. After selecting the ROI, Fiji was used to make a script to facilitate the copy of the ROIs to the other Dixon MR images. Also, collect the X, Y coordinates and the pixel value of the ROI to calculate the pancreatic fat percentage. Each pixel value was validated and calibrated against proton magnetic resonance spectroscopy (1

H-MRS) and a correction was applied. Any MRI fat signal above 20% is likely due to contamination by visceral fat tissue. Similarly, pixels almost devoid of fat (<1%) are likely to represent major pancreatic ducts or blood vessels, and these cannot be discriminated on the BTFE image (24). All the pixel values between 1-20% were used to calculate the average of the three parts of the pancreas. The mean of the three different regions and the mean of each individual pixel were also calculated.

Table 1- Demographics characteristics of the study population (n=832). Characteristics are described in mean, standard deviation, and percentages.

Demographics characteristics			
	NGM (n=283) n(%)	Prediabetes (n=282) n(%)	T2DM (n=267) n(%)
Age (years)	57 (±7.82)	61.82 (±8.23)	62.16 (±38.44)
Sex	114 (50.9)	139 (49.3)	134 (50.2)
Education Level			
Low	67 (23.7)	111 (39.4)	119 (44.6)
Medium	74 (26.3)	29.4 (29.9)	78 (29.2)
High	140 (49.8)	84 (29.8)	64 (24.0)
Smoking status			
Never	120 (42.4)	87 (30.9)	104 (39.0)
Former	126 (44.5)	157 (55.7)	127 (47.6)
Current	35 (12.4)	36 (12.8)	35 (13.1)
Alcohol use			
None	38 (13.4)	53 (18.8)	80 (30)
Low	173 (61.1)	144 (51.1)	142 (53.2)
High	70 (24.7)	84 (29.8)	43 (16.1)
Sum score Dutch healthy diet	85.07 (±15.12)	82.69 (±15.12)	81.82 (±15.25)
Total physical activity per week (hours/week)	14.21 (±8.47)	13.78 (±7.16)	12.47 (±7.10)
Healthy measures			
Total cholesterol (mmol/L)	5.40 (±0.98)	5.43 (±1.12)	4.63 (±1.03)
HDL cholesterol (mmol/L)	1.62 (±0.47)	1.49 (±0.42)	1.36 (±0.40)
LDL cholesterol (mmol/L)	3.229 (±0.89)	3.21 (±1.01)	2.50 (±0.89)
Triglycerides (mmol/L)	1.23 (±0.70)	1.65 (±0.93)	1.78 (±1.03)
Use of lipid-modifying drugs	32 (11.3)	94 (33.3)	184 (68.9)
Systolic blood pressure (mm/hg)	130.30 (±16.57)	135.99 (±16.65)	140.09 (±17.49)
Diastolic blood pressure (mm/hg)	74.64 (±9.89)	76.23 (±9.16)	76.21 (±9.54)

Measures of body			
Body mass index (kg m^2)	25.47 (±3.13)	27.77 (±4.36)	29.40 (±4.78)
Waist circumference (cm)	90.34 (±10.60)	96.87 (±11.66)	102.61 (±13.40)
Hip circumference (cm)	99.92 (±6.37)	102.95 (±9.06)	105.39 (±10.10)
Subcutaneous fat (cm)	200.76 (±75.78)	231.65 (±95.46)	248.08 (±101.14)
Visceral fat (cm)	135.44 (±80.20)	185.70 (±85.70)	234.08 (±103.19)
Liver fat (%)	3.68 (±4.34)	6.75 (±6.45)	8.68 (±7.01)
Trunk fat percentage (%)	31.95 (±6.94)	35.49 (±6.88)	37.45 (±7.11)
Subtotal body fat percentage (%)	32.89 (±7.83)	35.30 (±7.54)	36.75 (±7.53)
Total body fat percentage (%)	32.31 (±7.36)	34.63 (±7.16)	36.04 (±7.20)
Measures of glucose metabolism			
Fasting glucose (mmol/L)	5.13 (±0.41)	5.85 (±0.58)	7.84 (±1.93)
Post-Load glucose (mmol/L)	5.17 (±1.19)	8.14 (±1.56)	14.32 (±3.76)
HbA1c (%)	5.33 (±0.37)	5.66 (±0.39)	6.80 (±1.03)
Use of oral drugs for T2DM (n)	n/a	n/a	177 (66.3)
Use of insulin for T2DM (n)	n/a	n/a	47 (17.6)
Measures of Pancreatic fat			
Head (%)	6.79 (±3.55)	8.07 (±3.60)	9.04 (±3.57)
Body (%)	6.73 (±3.23)	8.16 (±3.09)	8.87 (±3.50)
Tail (%)	6.99 (±3.15)	8.03 (±3.40)	8.90 (±3.59)
Mean pancreas fat (%)	6.80 (±2.52)	7.95 (2.58)	8.67 (2.67)
Mean each pixel (%)	6.87 (±2.65)	8.14 ((±2.75)	8.95 (±2.76)

Assessments of body composition - T1-weighted images obtained by magnetic resonance imaging (MRI) were used to quantify adipose tissue area. Visceral fat area (cm2) and subcutaneous fat (cm2) were determined at the top level of the fourth lumbar vertebral body using a single-slice MRI-image in transverse plane. To quantify liver fat percentage, in and opposing phase MRI images were used to assess the differences in resonance frequencies between water and fat proton signals. Weight (kg) and height (cm) were measured using a scale and stadiometer (Seca, Hamburg,Germany). BMI (kg/m^2) was calculated by dividing weight by the square of the body height. The trunk fat percentage, subtotal body fat percentage and total body fat percentage were collected by assessed by dual-energy X-ray absorptiometry.

Assessments of covariates - All participants completed questionnaires regarding age, sex, educational level (low, medium, and high), smoking status (never, former, current smoker), and history of cardiovascular disease (CVD) (20). Use of medication was assessed during medication interviews. Daily activity levels were measured using the activPAL3 physical activity monitor (PAL Technologies, Glasgow, UK), as described elsewhere (24). Subsequently, total minutes of moderate to vigorous physical activity per day were calculated. Hip circumference, waist circumference, office systolic

and diastolic blood pressure were measured during a physical examination. Glycated hemoglobin A1c (HbA1c), and lipid were measured
in venous blood. Post-Load glucose (mmol/L) were determined by oral glucose tolerance test.

Statistic- All statistical analyses will be performed using SPSS version 25.0 software (IBM Corporation, Armonk, United States). Continuous variables are expressed in mean ± SD if normally distributed or median. On the other hand, categorical data are expressed as numbers and percentages compared with the chi-square test. Continuous variables will be assessed using the intra-class coefficient and the Bland-Altman plot with limits of agreement. Moreover, the association between pancreatic fat percentage and T2DM was assessed by univariate and multivariate linear regression models. The models were adjusted for confounders.

RESULTS

Individual's characteristics - Table 1 shows the characteristics of the overall population. Of the 832 participants in this study, 283 (34%) have a normal glucose metabolism, 282 (34%) have prediabetes and 267 (32%) have T2DM. The mean average of age of the NGM group is 57 (±7.82) years, prediabetes group 61.82 (±8.23) and for T2DM 62.16 (±38.44) years. Individuals of the T2DM group, were more often men, were older, had a lower educational level, lower sum score healthy diet and were less physically active than were those in the NGM group. Compared to participants with NGM and prediabetes, those with T2DM have a lower total cholesterol, HDL and LDL cholesterol levels and higher triglycerides levels, systolic blood pressure, diastolic blood pressure and higher use of lipid-modifying drugs. In the body composition measures of the three groups, T2DM have the highest BMI, waist, and hip circumference, subcutaneous fat, visceral fat, liver fat, trunk fat, subtotal body fat and total body fat percentage. The prediabetes group are between NGM and T2DM. Furthermore, the fasting glucose, post-load glucose, HbA1c levels are higher in the T2DM group. The prediabetes group have also higher fasting glucose, post-load glucose and HbA1c levels compared with the NGM group. Finally, the pancreatic fat percentage is the lowest in the NGM, this for the all-region of interest, the mean of the region of interest and the mean of the individual pixels. Participants with prediabetes have a higher mean of pancreatic fat, compared with NGM, not with T2DM. Diabetes group have the highest pancreatic fat percentage (figure 3).

Table 2- Linear regression model representing the association between the different regions of the pancreas and the average of the regions and each pixel value.

	β- coefficient [95% CI*]			
	Body	Tail	Mean ROI	Mean PIX
Head	**0.566 [0.50-0.63]**	**0.390 [0.32-0.46]**	**1.04 [0.98-1.11]**	**1.06 [1.01-1.11]**
Body		**0.48 [0.42-0.54]**	**1.05 [1.01-1.10]**	**1.00 [0.95-1.04]**
Tail			**0.91 [0.85-0.98]**	**0.94 [0.88-0.99]**
Mean ROI				**0.90[0.88-0.92]**

**CI: Confidence interval*
**ROI: Region of interest, PIX: pixel*
**Regression model was crude analysis*

Association between the different regions of interest, the mean of the regions of interest and mean of the pixel value of each ROI. - Table 2 shows the association between the different regions of interest, the mean of the regions of interest and mean of the pixel value of each ROI. The association between head and body is 0.566, the association between head and tail is 0.390. For body has an effect of 0.4777 on tail. The average of the three regions of interest has an influence of 1.042 on head, 1.054 on body and 0.914 of ROI 3. The mean of each pixel value has an association on head with 1.063 and 1.000 on body and 0.936 on tail. Moreover, the association between the average of the three regions has effect of 0.897 on the mean of each value. The analyses below are performed with ROI head, body, tail and Mean ROI, Mean Pixel. Although the results of head show the highest associations, the results of all these different parameters of pancreatic fat are comparable. Below the results of head are described. The other results are described in appendix. (Supplement table 1-4).

Relationship between and lifestyle factors and pancreatic fat –The linear regression analyses are presented in table 3. The results show an association between lifestyle factors (education level, total physical activity, alcohol, and Dutch healthy diet) and pancreatic fat percentage. For education, smoking, physical activity, and Dutch healthy diet was towards negative association, in contrast with alcohol, positive association.
However, this association was lost after adjustment for age, sex, prediabetes and T2DM (table 3, model 3).

Relationship between and adipocyte and pancreatic fat- In contrast, the adiposity depots are positively associated with pancreatic fat percentage, after full adjustment for potential factors. Similarly, for the anthropometric measures, the association between pancreatic fat percentage and anthropometric measures is positive associated, also after adjustment.

Association between (pre)diabetes and pancreatic fat percentage- Prediabetes and T2DM is significantly associated with pancreatic fat percentage, adjusted for age and sex (table 4, model 2). The associations remain significant, even after correcting lifestyle cofounders (table 4 model 3). In addition, the association between prediabetes and pancreatic fat percentage, are present after correcting for liver fat (table 4 model 4d). No significant relation was observed between the prediabetes and pancreatic fat percentage after adjustment for BMI, subcutaneous and visceral. For T2DM, there is an association between T2DM and pancreatic fat. The association disappear after adjusting visceral fat (table 4 model 4 a, b, and c).

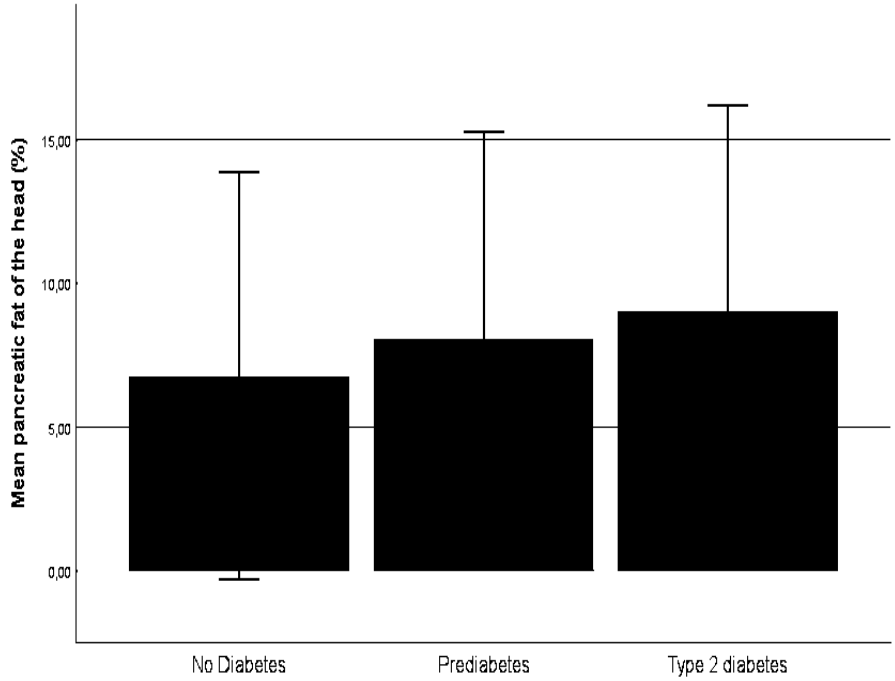

Fig. 3 – Pancreatic fat percentage of the pancreas head in the normal glucose metabolism, prediabetes and T2DM. *Present the mean of pancreatic fat of the head in the 3 groups: no diabetes, prediabetes and T2DM, with the standard deviation. T2DM have the highest pancreatic fat, compared with the prediabetes and no diabetes.*

Table 3 – Linear regression models representing the associations between pancreatic fat and lifestyle factors, MRI-derived measures of adipose tissue and anthropometric measures.

	β for pancreatic fat percentage (95%-CI)*
Lifestyle factors	
Education level	
Model 1	**-0.484 [-0.783- -0.184]**
Model 2	**-0.409 [-0.709- -0.109]**
Model 3	-0.250 [-0.550-0.050]
Smoking status	
Model 1	-0.123 [-0.508-0.261]
Model 2	-0.145 [-0.513-0.223]
Model 3	-0.189 [-0.550-0.172]
Total physical activity per week (hours/week)	
Model 1	**-0.050 [-0.085- -0.014]**
Model 2	**-0.035 [-0.069-0.000]**
Model 3	-0.026 [-0.060-0.008]
Alcohol total (g/day)	
Model 1	**0.017 [0.00-0.033]**
Model 2	-0.001 [-0.018-0.016]
Model 3	-0.002 [-0.014-0.19]
Sum score Dutch Healthy Diet	
Model 1	**-0.021 [-0.038- -0.004]**
Model 2	-0.016 [-0.033-0.002]
Model 3	-0.009 [-0.026-0.008]
MRI-derived measures of adipose tissue	
Subcutaneous adipose tissue (cm2)	
Model 1	**0.008 [0.005-0.010]**
Model 2	**0.012 [0.009-0.014]**
Model 3	**0.010 [0.008-0.013]**
Visceral adipose tissue (cm2)	
Model 1	**0.017 [0.014-0.019]**
Model 2	**0.015 [0.013-0.018]**
Model 3	**0.014 [0.011-0.017]**
Liver fat percentage (%)	
Model 1	**0.112 [0.073-0.152]**
Model 2	**0.111 [0.073-0.149]**
Model 3	**0.082 [0.042-0.122]**
Anthropometric measures	
Body Mass Index (kg/m2)	
Model 1	**0.290 [0.235-0.344]**
Model 2	**0.279 [0.226-0.331]**
Model 3	**0.252 [0.196-0.308]**
Waist circumference (cm)	
Model 1	**0.127 [0.109-0.145]**
Model 2	**0.116 [0.097-0.135]**
Model 3	**0.109 [0.088-0.130]**
Hip circumference (cm)	
Model 1	**0.109 [0.081-0.136]**
Model 2	**0.122 [0.095-0.148]**
Model 3	**0.107 [0.080-0.135]**

*Data are presented as regression coefficients (95% CI) and should be interpreted as the multiplicative change in the pancreatic fat content that is associated with the variables and pancreatic fat.

Model 1: Crude model

Model 2: + age, sex,

Model 3: + prediabetes and T2DM.

DISCUSSION

In this cross-sectional study, we describe no difference in percentage fat between the regions of interest The percentage fat between the ROIs is weakly associated with each other. Both prediabetes and diabetes are associated with pancreatic fat percentage, independent of sex, age, and liver fat. For prediabetes, the association attenuates after adding BMI, subcutaneous and visceral fat to the model. In comparison with T2DM, the association disappears after correcting for BMI and visceral fat.

Similar to our project, an earlier study describes that pancreatic fat is widespread on all the pancreas regions and not allocated to a specific region (25). Moreover, in this study, they found that the averages of the different ROIs are also similar to each other. The total study population was 70 patients with T2DM, a smaller group compared with our study population and used the same method for determine pancreatic fat. In contrast to our study, the literature reported that variability in fat content between the regions is present (21, 24). and showed that the pancreatic fat increased from the head to tail (21). The two studies had the same method, as our study, to detect pancreatic fat. The study population was smaller and consist only of T2DM (21,24).

For the lifestyle factors, we observed significant association between the lifestyle factors education level, total physical activity, alcohol, and Dutch healthy diet and pancreatic fat percentages. No relation between smoking and pancreatic fat is detected in our study. After the adjustment for age and sex, the association of alcohol and Dutch healthy diet with pancreatic fat disappeared, probably because they are highly associated with (pre)diabetes. The association of education level and physical activity was also significant after addition of age and sex to the model. This indicate that educational level and physical activity are independently of age and sex associated with pancreatic fat. Both educational levels, as proxy of economic status, and physical activity are well known factors effecting body composition.

As far as we know, no other literature is known between educational level and pancreatic fat. Educational level is often thought to be related to social economic status. In general, social economic status is related to healthy lifestyle. Even for the knowledge about physical activity and pancreatic fat is limited. It has been speculated, similar as T2DM, physical activity is an important factor in the development of pancreatic fat. Furthermore, one other manuscript describes to see no differences in the increase in pancreatic fat after exercise training. They investigated changes in pancreatic fat after physical activity in a study population of 83 individuals. It consisted of NGM, prediabetes and T2DM group and did training scheme of six exercise sessions over 2 weeks (26). The association of physical activity and education was attenuated after adding prediabetes and type 2 diabetes to the model. This indicates that physical activity and education are not independent of pre(diabetes) associated with pancreatic fat percentage. It is broadly excepted to both physical activity and education (social economic status) are highly associated with pre(diabetes). Moreover, the lifestyle factors, the results of alcohol were against the expectation. Alcohol only shows an association with pancreatic fat, in the crude model. The association completely attenuated after correction for age and sex. Based on the knowledge of alcohol and liver fat, it was expected that alcohol consumption ensures chronic pancreatitis, characterized by chronic inflammation, fibrosis, and destruction of exocrine and endocrine tissue (27). In the literature, similar results to our study are described, alcohol is related to pancreatic fat in univariate model, in the multivariable analyse the association is lost (28). Other studies indicate that alcohol is a risk factor in the development of fatty pancreas and therefore, they excluded the patients with a higher intake of >10 g/day in women and >20 g/day in men. However, they did not analyse or show the results of the association of alcohol and pancreatic fat. (13, 29, 30)

In contrast to the findings for lifestyle factors, adipose depots and the anthropometric measures are associated with pancreatic fat percentage, even after adjustment for age, sex and (pre)diabetes. Not many manuscripts use (pre)diabetes as independent variable as we did and describe the results in detail as we do, they investigate more in association between T2DM and pancreatic fat and the other cofounders than adipocyte (30, 31). Tirkes T et al. describe that subcutaneous fat is not related to pancreatic fat. They evaluated this effect in a study population of 186 with only T2DM patients (32). Miscellaneous results for visceral fat and the association with pancreatic fat are described. Tirkes T et al. find a result that increasing visceral fat is direct correlated with pancreatic fat. This study was performed on 118 study population with chronic pancreatitis and used 3-T MRI scanner to evaluate the pancreatic fat (32). However, another research group, who works with individuals with impaired fasting glucose and/or impaired glucose tolerance had the same result as our study (16). The different between the studies, they used a spectral–spatial fat selective MR-technique (16). In contrast, two research found the association between visceral fat and pancreatic fat, worked not with T2DM, and used both CT-scan. (30, 33).

In general, the literature describes a positive association as we do with liver fat, BMI, and waist circumference. Obesity-related complications of visceral fat are thought to be caused by a high lipolytic activity compared with subcutaneous fat. Visceral fat leads to more adipocytes between the organs and causing ectopic fat, defined as fat in other sites than adipose tissue such as the heart and pancreas (10). Moreover, visceral fat increases the flux of free fatty acids into the liver. This leads to intake of free fatty acids by hepatocytes and results in decreased insulin sensitivity and insulin resistance development. This mechanism could be similar in the pancreas, where visceral fat ensures the intake of free fatty acids, and develops a fatty pancreas, and leads to β-cell dysfunction, which could lead to T2DM (16-18).

In the present study, we confirm data in the literature that describe that T2DM individuals have higher pancreatic fat, compared to prediabetes and NGM (32, 34). Ahbab et al. found also that prediabetes group have a higher pancreatic fat than NGM (34). Our result shows that the association between (pre)diabetes and pancreatic fat is independent of age, sex, and lifestyle factors (education level, smoking, total physical activity, alcohol, and Dutch healthy diet). Moreover, the association in prediabetes is also independent of

Table 4- Linear regression models representing the associations between (pre)diabetes and pancreatic fat

	β for prediabetes (95%-CI)*
	Glucose metabolism status
	Prediabetes
Model 1	**1.286 [0.888-1.884]**
Model 2	**1.000 [0.350-1.649]**
Model 3	**0.902 [0.244-1.561]**
Model 4A	0.291 [-0.296-0.877]
Model 4B	0.485 [-0.102-1.07]
Model 4C	0.289 [-0.291 -0.871]
Model 4D	**0.640 [0.028-1.252]**
	T2DM
Model 1	**2.253 [1.645-2.860]**
Model 2	**1.854 [1.186-2.522]**
Model 3	**1.630 [0.936-2.324]**
Model 4A	**0.806 [0.182-1.431]**
Model 4B	**1.204 [0.595-1.81]**
Model 4C	0.515 [-0.123-1.152]
Model 4D	**1.411 [0.769-2.053]**

*****Data are presented as regression coefficients (95% CI) and should be interpreted as the multiplicative change in the pancreatic fat content that is associated with the (pre)diabetes and pancreatic fat.*

Model 1: Crude model
Model 2: + age, sex,
Model 3: + lifestyle factor
Model 4a: age, sex, and BMI
Model 4b: age, sex, and subcutaneous fat
Model 4c: age, sex, and visceral fat
Model 4d: age, sex, and liver fat

liver fat, but not independent of BMI, subcutaneous and visceral fat. In T2DM the association is independent of BMI, liver fat and subcutaneous, but not from visceral fat. These results are in line with the study of Guglielmi V showing an association between pancreatic steatosis and incidence of T2DM that disappeared after adjustment for potential confounders including BMI and liver attenuation (35). There have been few previous studies on the association with pancreatic fat. The results of one study were that non-alcoholic fatty pancreas disease was independently correlated with both prediabetes and type 2 diabetes adjusted for age (34). Moreover, one research group found that pancreatic fat is related to T2DM, in a study group of 126 male volunteers and with age between 25-70 years (36). Previous studies investigated in impaired insulin secretion or β-cell function (16, 19, 37). Our data and the data of others suggests that fat depots and (pre)diabetes are important factors in the levels of pancreatic fat. The consequences of fat accumulation in the pancreas might provoke to a decline β-cell mass and function. This can lead to rapid progression to diabetes. Another hypothesize is that T2DM and pancreatic fat could have no causative relation with each other and could be both an obesity-related complication. (34, 38, 39).

Limitations- This study has some limitations. The study has a cross-sectional design. It does not give the chance to research a causative relation between (pre)diabetes and pancreatic fat. The sample size was small and could not present the Dutch population. A better understanding of the relation could be achieved by a larger study population and a longer follow-up to understand the clinical relevance of (pre)diabetes and pancreatic fat. However, the group was balanced, consist of individuals with NGM, prediabetes and T2DM. Also, with approximately in each group equal men and women.

Future perspectives – More research is requested to understand a causative relation between (pre)diabetes and pancreatic fat. This dysfunction in insulin release might be caused by pancreatic fat and can affect the development of prediabetes and T2DM. If pancreatic fat reflects (or causes) β-cell dysfunction, this could be a possible hallmark that enables detecting (pre)diabetes in an early stage

CONCLUSION

T2DM has the highest pancreatic fat percentage, compared with NGM and prediabetes. Also, fat accumulation was present on the head, body, and tail of the pancreas. The association between (pre)diabetes and pancreatic fat was present after correcting for age and sex. The association for prediabetes, is dependent on BMI, subcutaneous and visceral fat. In the association with T2DM and pancreatic fat, BMI and visceral fat are important confounders. Further research is needed to explore the relation of pancreatic fat and insulin resistance and secretion in more detail.

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SUPPLEMENT

Table 1- Linear regression models representing the associations between (pre)diabetes and the fat percentage of the **body of the pancreas**

	β for prediabetes (95%-CI)
Glucose metabolism status	
Prediabetes	
Model 1	1.438 [0.893-1.983]
Model 2	1.201[0.601-1.801]
Model 3	1.139 [0.532-1.745]
Model 4A	0.801 [0.248-1.355]
Model 4B	0.904 [0.357-1.450]
Model 4C	0.757 [0.209-1.305]
Model 4D	0.836 [0.273-1.398]
T2DM	
Model 1	2.139 [1.583-2.694]
Model 2	1.704 [1.084-2.324]
Model 3	1.562 [0.920-2.203]
Model 4A	1.221 [0.631-1.812]
Model 4B	1.405 [0.836-1.975]
Model 4C	0.972 [0.370-1.574]
Model 4D	1.336 [0.741-1.930]

**Data are presented as regression coefficients (95% CI) and should be interpreted as the multiplicative change in the pancreatic fat content that is associated with the (pre)diabetes and pancreatic fat.*
Model 1: Crude model
Model 2: + age, sex,
Model 3: + lifestyle factor
Model 4a: age, sex and BMI
Model 4b: age, sex and subcutaneous fat
Model 4c: age, sex and visceral fat
Model 4d: age, sex and liver fat

Table 2- Linear regression models representing the associations between (pre)diabetes and the fat percentage of **the tail of the pancreas**

	β for prediabetes (95%-CI)
Glucose metabolism status	
Prediabetes	
Model 1	1.041 [0.464-1.617]
Model 2	0.819 [0.183-1.456]
Model 3	0.730 [0.086-1.375]
Model 4A	0.629 [0.702-1.984]
Model 4B	0.668 [0.076-1.260]
Model 4C	0.515 [-0.080-1.110]
Model 4D	0.836 [0.741-1.930]
T2DM	
Model 1	1.917 [1.326-2.590]
Model 2	1.639 [0.978-2.301]
Model 3	1.467 [0.783-2.151]
Model 4A	1.343 [0.030-1.227]
Model 4B	1.422 [0.801-2.043]
Model 4C	1.042 [0.386-1.697]
Model 4D	1.36 [0.273-1.398]

**Data are presented as regression coefficients (95% CI) and should be interpreted as the multiplicative change in the pancreatic fat content that is associated with the (pre)diabetes and pancreatic fat.*
Model 1: Crude model
Model 2: + age, sex,
Model 3: + lifestyle factor
Model 4a: age, sex and BMI
Model 4b: age, sex and subcutaneous fat
Model 4c: age, sex and visceral fat
Model 4d: age, sex and liver fat

Table 3- Linear regression models representing the associations between (pre)diabetes and the fat percentage **of the mean of the regions of interest**

	β for prediabetes (95%-CI)
Glucose metabolism status	
Prediabetes	
Model 1	1.271 [0.821-1.720]
Model 2	1.036 [1.205-2.210]
Model 3	0.957 [0.464-1.451]
Model 4A	0.610 [0.166-1.053]
Model 4B	0.709 [0.868-1.780]
Model 4C	0.548 [0.112-0.984]
Model 4D	0.722 [0.266-1.178]
T2DM	
Model 1	2.078 [1.622-2.534]
Model 2	1.708 [1.205-2.210]
Model 3	1.524 [1.004-2.044]
Model 4A	1.121 [0.650-1.593]
Model 4B	1.324 [0.270-1.148]
Model 4C	0.827 [0.350-1.305]
Model 4D	1.406 [0.927-1.885]

**Data are presented as regression coefficients (95% CI) and should be interpreted as the multiplicative change in the pancreatic fat content that is associated with the (pre)diabetes and the mean of the regions of the pancreatic fat.*
Model 1: Crude model
Model 2: + age, sex,
Model 3: + lifestyle factor
Model 4a: age, sex and BMI
Model 4b: age, sex and subcutaneous fat
Model 4c: age, sex and visceral fat
Model 4d: age, sex and liver fat

Table 4- Linear regression models representing the associations between (pre)diabetes and **the mean of the pixels of the pancreas**

	β for prediabetes (95%-CI)
Glucose metabolism status	
Prediabetes	
Model 1	1.156 [0.729-1.583]
Model 2	0.946 [0.521-1.371]
Model 3	0.914 [0.440-1.388]
Model 4A	0.592 [0.167-1.016]
Model 4B	0.669 [0.248-1.089]
Model 4C	0.520 [0.102-0.938]
Model 4D	0.683 [0.248-1.119]
T2DM	
Model 1	1.872 [1.439-2.305]
Model 2	1.58 [1.097 – 2.060]
Model 3	1.38 [0.887-1.886]
Model 4A	1.045 [0.594-1.496]
Model 4B	1.215 [0.779-1.651]
Model 4C	0.756 [0.299-1.214]
Model 4D	1.297 [0.840-1.755]

**Data are presented as regression coefficients (95% CI) and should be interpreted as the multiplicative change in the pancreatic fat content that is associated with the (pre)diabetes and the mean of each individual pixels of the pancreatic fat.*
Model 1: Crude model
Model 2: + age, sex,
Model 3: + lifestyle factor
Model 4a: age, sex and BMI
Model 4b: age, sex and subcutaneous fat
Model 4c: age, sex and visceral fat
Model 4d: age, sex and liver fat