Abdominal Fat Depots are Related to Lower Cognitive Functioning and Brain Volumes in Middle-aged Males at High Alzheimer's Risk

Abdominal Fat, Cognition, and Brain Volumes

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What is already known about this subject?

- Alzheimer's dementia (AD) research is shifting towards the identification of populations at high risk to facilitate disentangling AD underlying mechanisms and novel treatment targets.
- Obesity is a risk factor for lower cognitive functioning and higher dementia risk, with different associations between sexes.
- Obesity is measured usually by body mass index (BMI), which poorly represents body fat distribution, and does not necessarily account for sex differences.

What are the new findings in your manuscript?

- High BMI was associated with high hepatic and pancreatic fat %, but not with visceral adipose tissue (VAT) %. In females only, high BMI was associated with high subcutaneous adipose tissue (SAT) %.
- Among middle-aged males at high AD risk, higher pancreatic fat % was associated with lower cognitive function and inferior frontal gyrus volume.
- VAT % and SAT % were inversely associated with middle frontal and superior frontal gyrus volumes in males and females.

How might your results change the direction of research or the focus of clinical practice?

- Abdominal fat depots, rather than BMI, will be assessed as a risk factor for lower cognitive functioning and higher dementia risk.

- Since to our knowledge, we are the first to do so, more research needs to be done regarding the association of pancreatic fat %, cognitive functioning, and brain volumes.
- Future investigation of the underlying mechanisms that may explain the observed associations, may lead to sex-specific interventions for the promotion of brain health.

Abstract

Objective: High body mass index (BMI), which poorly represents specific fat depots, is linked to poorer cognition and higher dementia risk, with different associations between sexes. We examined associations of abdominal fat depots with cognition and brain volumes and whether sex modifies this association.

Methods: 204 healthy middle-aged Alzheimer's-dementia (AD) offspring (mean age=59.44, 60% females) underwent abdominal magnetic resonance imaging to quantify hepatic, pancreatic, visceral (VAT), and subcutaneous adipose tissue (SAT), assessment of cognition and brain volumes.

Results: In the whole sample higher hepatic fat % was associated with lower total grey matter volume (β=-0.17, $p<0.01$). Primarily in males, higher pancreatic fat % was associated with

lower global cognition (Males: β=-0.27, p=0.03; Females: β=0.01, p=0.93) executive function (Males: β=-0.27, p=0.03; Females: β=0.02, p=0.87), episodic memory (Males: β=-0.28, p=0.03; Females: β=0.07, p=0.48) and inferior frontal gyrus volume (Males: β=-0.28, p=0.02; Females: β=0.10, p=0.33). VAT and SAT were inversely associated with middle frontal and superior frontal gyrus volumes in males and females.

Conclusion: In middle-aged males at high AD-risk, but not in females, higher pancreatic fat, was associated with lower cognition and brain volumes. These findings suggest a potential sex-specific link between distinct abdominal fat with brain health.

Introduction

With increasing life expectancy, new global health issues are emerging, including the accelerating prevalence of neurodegenerative diseases. The World Health Organization reported in March 2023 that 55 million people are affected by dementia worldwide and every year, there are nearly 10 million new dementia cases, with Alzheimer's dementia (AD) being the most common form[1]. In addition to the direct impact on affected individuals and their families, there is a high societal and economic impact of the disease. Treatments approved for AD are of marginal efficacy and have small effects on disease progression[2,3] – possibly because the pathological changes occur long before the actual appearance of the symptoms[4]. Therefore, the identification of populations at high risk[5] to facilitate disentangling AD underlying mechanisms and novel treatment targets is a public health urgency.

There is broad evidence indicating an association of midlife obesity with greater dementia risk in late life[6,7]. Midlife obesity is one of nine modifiable risk factors for dementia with a 1.6-fold risk compared to non-obese individuals[5]. However, most of the research on the relationship between obesity, AD, and cognitive decline has been based on body mass index (BMI) and waist circumference, which poorly reflect body fat distribution[8]. Fat is stored in different ways in the body such as subcutaneous adipose tissue (SAT), visceral adipose tissue (VAT), and ectopic fat. SAT is stored beneath the skin while VAT is stored around the abdominal organs. VAT and SAT have been found to have different associations with both cognitive functioning and brain volumes. Higher VAT was associated with lower cognitive function[9], lower cortical thickness[10], and smaller brain volumes[10,11] in older adults. SAT was associated with worsening cognitive function in men[12], while it was found to be protective among women[13].

Ectopic fat deposition, which usually occurs due to excess visceral adiposity, is a harmful condition where lipids accumulate in normally lean tissues such as the heart, liver, and pancreas. Here, we quantified ectopic hepatic and pancreatic fat %, both related to metabolic syndrome[14]. Although associations of hepatic fat % with cognitive functioning and brain pathologies have rarely been studied, there is evidence for associations of nonalcoholic fatty liver disease (NAFLD), with lower cognitive scores[15] and AD[16]. There is limited knowledge about the role of pancreatic fat % in cognitive functioning or neuropathology.

Sex differences in human adipose tissues' distribution are well established. In general, females have more fat, specifically more SAT, while they are characterized by lower VAT mass compared to males[17]. Assessment of ectopic fat reveals higher hepatic and muscle fat in males while females had lower limb fat[18]. However, little is known about whether there are sex differences in the associations of fat depots with brain aging and cognition.

In the current study, our objective was to first, examine associations of abdominal fat depots (hepatic, pancreatic, VAT, and SAT) measured by MRI, with cognitive functioning and AD-related brain volumes in middle-aged participants at high AD risk due to parental family history. Second, we investigated whether these associations differed in males and females.

Methods

Participants

The study sample is based on the Israel Registry for Alzheimer's Prevention (IRAP) cohort study, which is comprehensively described elsewhere[19]. Briefly, this study is a collaboration between the Sheba Medical Center, Israel, the Icahn School of Medicine at Mount Sinai, NY, and the Maccabi Healthcare Services (MHS), the second-largest health maintenance organization in Israel. The study follows cognitively asymptomatic middle-aged offspring of AD patients. Parental diagnosis of AD is validated by medical charts and the Dementia Questionnaire[20]. Participants are aged 40-65 at baseline and must be fluent in Hebrew. Medical, laboratory, and pharmacy data are available for all IRAP participants through MHS electronic medical charts. Each IRAP participant completes an entry core assessment and follow-up assessments approximately 3 years apart. The IRAP study has 420 participants. Of them, 315 were randomly approached for abdominal MRI scans and 204 agreed to participate

in the abdominal fat depots component of the study and were eligible for MRI. Of these, 142 participants underwent a structural volumetric brain MRI scan.

Anthropometric and clinical variables

On the day of the cognitive assessment, blood pressure and anthropometric measures were obtained by the study staff according to the Atherosclerosis Risk in Communities Study (ARIC) protocols. Participants were instructed to sit for 10 minutes and then have blood pressure readings obtained. The cuff size was chosen according to the participant's arm circumference. Blood pressure was measured three times within an examination visit with the participant seated using a random-zero sphygmomanometer. The average of the second and third readings were used for both systolic and diastolic blood pressure. BMI was calculated as weight in kilograms (kg) divided by height in squared meters (m^2). Weight was measured to the nearest tenth of a kg using a balance scale. Height was measured to the nearest centimeter using a wall-mounted ruler. Type 2 diabetes (T2D) diagnosis was received through the MHS diabetes registry and smoking status was collected at baseline by the IRAP team.

Laboratory testing

Fasting blood samples (overnight fast) were collected at baseline and all follow-up assessments. Bloods were processed using standard methods in the Sheba main laboratory. Laboratory assessments included total cholesterol and HbA1c.

Neuropsychological assessment

IRAP participants completed a comprehensive neuropsychological battery at baseline and all follow-up assessments. This battery includes tests assessing multiple cognitive functions. Factor analysis summarizes the neuropsychological measures into four domains: (1) episodic memory [Rey Auditory Verbal Learning Test (RAVLT) immediate recall (sum of tests 1-5), RAVLT delayed recall and RAVLT recognition]; (2) executive function (Trails making test A, Trails making test B, Block design, and Digit Symbol); (3) working memory (digit span forward, digit span backward, and letter-number sequencing); (4) language (verbal IQ similarities, verbal IQ vocabulary, and the average of phonemic and animal fluency); and (5) global cognition which was calculated as the average of the z-scores of the four cognitive domains. The neuropsychological test scores were transformed into Z scores (reversing time-based scores—Trails A and Trails B—so high scores represent better performance) and averaged

for each domain[19].

Abdominal Adiposity MRI acquisition

Well-validated methods[21,22] were used. A 15-minute (min) scan on a 3 Tesla Philips Ingenia scanner included five sequences: 1+2) free-breathing axial navigator-triggered T2-weighted turbo spin-echo (TSE) with/without fat suppression; 3) coronal breath-hold T2-weighted TSE sequence with two echoes; Images were acquired within multiple short (15 seconds) breath-holds to avoid motion artifacts. Two additional sequences that use the breath-hold technique were performed as follows: 4) 2D sequence generated standard in-phase (IP) and opposed-phase or out-phase (OP) image sets. 2D axial dual-echo T1-weighted spoiled gradient-echo sequence with a repetition time (TR)=121 milliseconds (ms), OP/IP echo time (TE) 1.2/2.3 ms, flip angle 55°, half-scan factor 0.85, slice thickness=5 millimeter (mm), a field of view (FOV) 400 mm×250mm×241.5, matrix 252×205; 5) 3D modified DIXON (mDIXON)[23] imaging technique with a multi-echo 2-excitation pulse sequence for the phase-sensitive encoding of fat and water signals with TR= 5.6 ms; TE 1=0.97 ms; TE 2=0.7 ms; FOV 400×350×252 mm; voxel size 2.5×2.5×6 mm.

Quantitative Abdominal Adiposity image analysis

Abdominal fat depots were assessed by 2 raters using Philips IntelliSpace Portal (Philips Medical Systems) and SegmentGUI[22].

Hepatic fat**:** Hepatic fat percentage was quantified using a region of interest (ROI) approach, as in previous studies[24]. 1-3 non-overlapping circular ROIs of 200 mm^2 in the area were drawn on the OP image on each liver segment. Care was taken to ensure they include liver parenchyma only and devoid of large vessels, ducts, organ boundaries, focal hepatic lesions, and imaging artifacts. ROIs were copied to the IP and mDIXON Quant fat-fraction (FF) images. The fat mean percentage was calculated for each segment and the liver as a whole. This method has a high inter and intra-reader agreement for hepatic fat quantification.

Pancreatic fat: Pancreatic fat percentage was quantified using an ROI approach[21]. 3 non-overlapping circular ROIs of 100 mm^2 in the area were drawn on OP images in the pancreatic head (caput), body (corpus), and tail (cauda) in three successive MRI slices. Care was taken to ensure they included pancreatic parenchyma only and were devoid of large vessels, ducts,

organ boundaries, focal pancreatic lesions, and imaging artifacts. The mean fat percentage was calculated for each part of the pancreas and the pancreas as a whole.

VAT and SAT quantification: VAT and SAT were quantified using SegmentGUI – a MATLAB-based semiautomatic software[22]. To do this, tissues were manually categorized into color-coded groups, which included SAT, VAT, peri-muscular fat (fat within and around the latissimus dorsi and diaphragm), and non-classified fat (fat surrounding the vertebrae and fat deposits not associated with any of the aforementioned tissues) (Figure 1). To select the specific fat mass area, a semi-automatic approach was employed, which involved connected pixels, combined with various manual tools like rectangles, circles, polygons, or freehand drawing for finer adjustments and corrections when necessary. The quantification of fat mass regions was expressed as a proportion (percentage) of the total area encompassing all types of fat. Mean VAT and SAT percentages were calculated from four axial slices, specifically at the L2-L3, L3-L4, L4-L5, and L5-S1 levels.

Figure 1: Representative image of SegmentGUI. Abdominal fat tissues were manually categorized into color-coded groups, which included SAT (purple and light blue), VAT (green), peri-muscular fat (pink), and non-classified fat (red).

Structural brain MRI imaging acquisition

All IRAP participants were invited to undergo a structural brain MRI scan. Those who agreed and did not have contraindications (e.g., claustrophobia, carriers of metallic grafts, or pacemakers) were invited to the Sheba Medical Center Division of Diagnostic Imaging. Scans were performed using a 3 Tesla scanner (GE, Signa HDxt, v16VO2) equipped with an eight-channel head coil. High-resolution (1 mm^3) images were acquired using a 3D inversion recovery prepared spoiled gradient-echo (FSPGR) T1-weighted sequence (TR/TE = 7.3/2.7s, 20^o flip angle, TI 450 ms).

Volumetric brain image analysis

T1-weighted image data were pre-processed using the CAT12 toolbox[25] (http://dbm.neuro.uni-jena.de/cat), an extension to the SPM12 software package (http://www.fil.ion.ucl.ac.uk/spm/software/spm12) in the MATLAB environment (The MathWorks, Inc., Natick, Massachusetts, United States). Gray matter (GM), white matter, and

cerebrospinal fluid components were obtained to calculate the total intracranial volume (TIV) in the native space. Neuromorphometrics atlas was used to extract specific regions of interest - Total GM, and AD-related ROIs including the hippocampus, inferior frontal gyrus (IFG), middle frontal gyrus (MFG), and superior frontal gyrus (SFG) were extracted from the smoothed modulated and normalized images and were entered into group comparisons adjusting for TIV. ROIs were selected a priori based on previous work from our group showing associations of smaller volumes of IFG and middle temporal gyrus with high BMI[26].

Statistical Analyses

T-tests or chi-square were used to compare males' and females' characteristics. ANCOVA was used to compare males' and females' brain volumes, controlling for TIV. Associations of abdominal fat depots (hepatic, pancreatic, VAT, and SAT %) with BMI were examined using linear regression, controlling for age, sex, education, and the time between assessments. Analyses were first performed for the whole sample, and then separately for males and females. Primary analyses were linear regressions performed to examine the association between abdominal fat depots and cognitive functioning (episodic memory, working memory, executive function, language, and global cognition), total GM volume, and AD-related brain volumes (hippocampus, IFG, MFG, and SFG). Analyses were first performed for the whole sample, and then separately for males and females. In model 1, we controlled for sociodemographic variables (age, years of education, sex), BMI, and time between assessments. In model 2, the following covariates were added: cardiovascular risk factors (total cholesterol, systolic and diastolic blood pressure, hemoglobin A1c [HbA1c]) which were measured on the day of cognitive assessment, and smoking status [never/past/current]) which was also assessed on the day of cognitive assessment. To adjust for variability in brain volume, TIV was included in all brain MRI analyses. Since T2D is highly related to fat, an additional exploratory model (model 3) included model 1 covariates and T2D status (yes/no). We then examined the interactions of sex with each of the fat measures on each of the cognitive and brain measures. It has been demonstrated that studies investigating interactions, especially when comparing between the sexes[27], can profit in terms of power by raising the Type I error rate from 5% up to 20% to detect interactions that would otherwise remain uncovered[28]. Therefore, in this study, we used a p-value of 0.1 for the interactions.

Results

Description of the sample: The sample included 204 IRAP participants with cognitive assessments and abdominal fat MRI. Among them, a subsample of 142 participants (69.6%) had a structural brain MRI scan. The subsample of brain MRI shared similar characteristics with the whole sample. There were no significant differences between males and females in demographic characteristics, BMI, and T2D occurrence (Table 1). Males had lower total cholesterol levels, ($p<0.001$), and higher systolic and diastolic blood pressure ($p<0.001$). Generally, males had higher pancreatic fat % ($p=0.05$) and more VAT % compared to females, while females had higher SAT% ($p<0.001$). There were no significant differences between males and females in global cognition, but males performed worse in episodic memory and better in working memory. Males had larger total GM and regional brain volumes compared to females ($p<0.001$).

Table 2 presents the associations of BMI with abdominal fat depots. High hepatic and pancreatic fat % were associated with high BMI in both males ($\beta=0.29$, $p=0.02$; $\beta=0.38$, $p=0.00$; respectively) and females ($\beta=0.35$, $p<0.00$; $\beta=0.38$, $p<0.00$; respectively); VAT% was not associated with BMI in either sex, while high SAT % was associated with high BMI in females ($\beta=0.23$, $p=0.01$) but not in males ($\beta=0.04$, $p=0.75$).

Associations of abdominal fat depots with brain volumes and cognitive functioning

Hepatic fat: Linear regression results of hepatic fat with cognitive functioning and brain volumes are presented in Table 3A. Hepatic fat was not associated with cognitive functioning in either males or females, irrespective of the covariates included. Hepatic fat % association with lower total GM volume approached significance in both males ($\beta=-0.23$, $p=0.02$) and females ($\beta=-0.18$, $p=0.02$) in model 1. However, in the model adjusting for cardiovascular risk factors (model 2) – the associations were no longer significant. Hepatic fat % was not associated with other regional brain volumes.

Pancreatic fat: In the entire sample, there were no associations between pancreatic fat % and cognition in either model. In model 1, high pancreatic fat % association with global cognition approached significance mainly in males (Males: $\beta=-0.27$, $p=0.03$; Females: $\beta=0.01$, $p=0.93$; p for interaction=0.10; Table 3B, Figure 2E). Similarly, higher pancreatic fat % was associated

with lower executive function in males but not in females (Males: β=-0.27, p=0.03; Females: β=0.02, p=0.87; p for interaction=0.08; Table 3B, Figure 2C). Adjustments for cardiovascular risk factors in model 2 were essentially unchanged both for global cognition and executive function (Table 3B). In model 1, high pancreatic fat % was associated with lower episodic memory function in males but not in females (Males: β=-0.28, p=0.03; Females: β=0.07, p=0.48; p for interaction=0.05; Table 3B, Figure 2A). These differences were attenuated after adjustment for cardiovascular risk factors (Table 3B). Pancreatic fat % was not related to working memory (Table 3B; Figure 2B) or language (Table 3B; Figure 2D).

In the whole sample, pancreatic fat % was not associated with any of the brain volume measures (Table 3B). In model 1, there was an association between high pancreatic fat % and lower hippocampal volume mostly in females (Males: β=0.01, p=0.91; Females: β=-0.28, p=0.01; p for interaction= 0.05; Table 3B, Figure 3B) with similar results after adjustment for cardiovascular risk factors (Table 3B). In model 1, higher pancreatic fat % was associated with lower IFG volume in males (β=-0.28, p=0.02) but not in females (β=0.10, p=0.33) and the interaction was significant (p=0.02) (Table 3B, Figure 3C). However, the differences were attenuated by the adjustment of cardiovascular risk factors (Table 3B). Pancreatic fat % was not related to total GM, MFG, and SFG volumes (Table 3B, Figures 3A, 3D, and 3E).

Figure 2: Scatterplots depicting different associations of pancreatic fat % with cognitive functioning in males (blue) and females (purple). Each line is representative of the results of the linear regression of model 1. Model 1 covariates: age, sex, education, the time between assessments, and BMI. The p-value of the interaction between pancreatic fat and sex (male/female) on cognitive functioning is presented.

Figure 3: Scatterplots depicting different associations of pancreatic fat % with volume of brain regions in males (blue) and females (purple). Each line is representative of the results of the linear regression of model 1. Model 1 covariates: age, sex, education, the time between assessments, BMI, and TIV. The p-value of the interaction between pancreatic fat and sex (male/female) on AD-related brain volumes is presented. Abbreviations: GM, Grey matter; cm, centimeters; IFG, inferior frontal gyrus; MFG, middle frontal gyrus; SFG, superior frontal gyrus.

VAT: VAT % was not associated with cognitive functioning in the full sample or in males and

females separately. The association of VAT% with SFG volume approached significance in model 1 where in males high VAT% was associated with higher SFG while in females this association was inverse (Males: β=0.23, p=0.05; Females: β =-0.16, p=0.09; p for interaction=0.01; Table 4A, Figure 4A). Additional adjustments for cardiovascular risk factors did not substantively change the results (Table 4A). Additionally, in males, in model 2, higher VAT % was associated with higher MFG volume (Males: β=0.29, p=0.03; Females: β=0.11, p=0.34; p for interaction=0.33; Table 4A). VAT % was not related to GM, Hippocampus, or IFG.

SAT: Like VAT %, SAT % was not associated with cognitive functioning in the full sample or in males and females separately. High SAT% had different trends of associations with SFG volume in males and females in model 1 (Males: β=-0.13, p=0.27; Females: β=0.13, p=0.21; p for interaction=0.09; Table 4B, Figure 4B) and 2 (Males: β=-0.20, p=0.14; Females: β=0.12, p=0.12; p for interaction=0.07; Table 4A). In males, in model 2, higher SAT % was associated with lower MFG volume (Males: β=-0.27, p=0.03; Females: β=-0.07, p=0.56; p for interaction=0.25; Table 4B). SAT % was not related to GM, Hippocampus, or IFG.

Figure 4: Scatterplots depicting different associations of VAT and SAT % with SFG volume in males (blue) and females (purple). Each line is representative of the results of the linear regression of model 1. The p-value of the interaction between pancreatic fat and sex (male/female) on cognitive functioning is presented. Model 1 covariates: age, sex, education, the time between assessments, BMI, and TIV. Abbreviations: SFG, superior frontal gyrus; cm, centimeters.

Discussion

There is a growing body of literature that supports the idea that obesity is associated with brain health outcomes. Previous research has shown that obesity, primarily measured by BMI, is linked to lower GM volume, decreased white matter integrity, and decreased cognitive functioning[29,30]. However, less is known about the specific associations between abdominal fat depots and brain health outcomes. In this study of 204 middle-aged offspring of AD patients from the IRAP study[19], we examined the associations of four fat depots – hepatic, pancreatic, VAT, and SAT measured by MRI, with cognitive functioning and brain volumes. The volumetric

regions assessed in this study were chosen a priori. Specifically, we have found that long-term trajectories of obesity (based on BMI), in older adults with T2D, are associated with smaller volumes of the middle temporal gyrus and IFG, which underlies functions of decision-making, attention, and language[26]. In addition, a meta-analysis presented diverse associations of IFG and MFG volume with obesity[31]. Consistent with these results, in the current middle-aged cohort we found associations of abdominal fats with hippocampal, IFG, and MFG volumes suggesting that these brain regions may be particularly sensitive to the deleterious effects of fat. Overall, our results show that higher pancreatic fat is associated with lower cognitive functioning and lower IFG volume primarily in males, while higher pancreatic fat % is associated with lower hippocampal volume mostly in females. Higher VAT % was related to higher SFG and MFG volumes in males only, while higher SAT% was related to lower MFG volume, also in males only. We have chosen to use fat percentage in order to avoid the limitation of different abdominal circumferences. We repeated the analyses using fat area (data not shown) and results remained essentially unchanged. We also replaced BMI with waist circumference (data not shown) and again, results remained similar. Finally, hepatic fat was not associated with brain volumes or cognition in neither males nor females. These results suggest that already in midlife, abdominal fat accumulation may have deleterious effects on brain health, especially in men.

We believe that this is the first study examining the relationships of pancreatic fat with brain volumes and cognition in middle-aged adults. Pancreatic fat accumulation leads to hampered insulin secretion[32] which in turn, has been associated with AD-related neuropathology and cognitive functioning[33]. Here we show that pancreatic fat accumulation is related to lower episodic memory, primarily in males. Pancreatic fat was also associated with IFG volume in men, consistent with our prior findings of associations of long-term obesity with a smaller volume of IFG[26]. Given the association between abdominal fat infiltration and T2D, we anticipated that considering T2D in the analysis would weaken the relationships between fat accumulation and brain/cognitive functions. However, to our surprise, adjusting for T2D, as outlined in Table S1, did not substantially change these associations. It is worth noting that only 7% of participants had T2D, which may have been an insufficient number to exert a significant mediating effect. In this sample, females had lower pancreatic fat % than males. Earlier studies reported that increased levels of pancreatic fat may lead to the development of

β-cell dysfunction and insulin resistance[34] and that a greater amount of visceral and hepatic adipose tissue, in conjunction with the lack of a possible protective effect of estrogen, may be related to higher insulin resistance in males compared with females[35]. Including cardiovascular covariates in the regression model (smoking status, total cholesterol, HbA1c, diastolic and systolic blood pressure) attenuated the interaction of sex with pancreatic fat on episodic memory and IFG volume (Table 3) suggesting a potential mechanistic pathway between fat, brain, and cognition, *via* cardiovascular risk and disease. Although there are no medications that address specific fat depots, weight loss, using diet or exercise, has been related to a decrease in pancreatic fat accumulation in males, and females[36].

While an association of pancreatic fat with IFG was found predominantly in males, higher pancreatic fat % was associated with lower hippocampal volume, mainly in females. The hippocampus is the first region affected by AD[37], which is more prevalent in females[38]. This is consistent with recent findings from postmortem tissue of AD patients and normal controls, using integrated omics analysis on the hippocampus; the downregulation of the insulin signaling pathway in AD patients was significantly more pronounced in women than in men[39]. These findings may suggest that the vulnerability of the hippocampus to damage due to the downstream effects of pancreatic dysfunction including defective insulin release, possibly due to high pancreatic fat, is greater in women. Further research to disentangle sex effects on the role of pancreatic fat in hippocampal damage is warranted.

NAFLD is the term for a range of conditions caused by a build-up of fat in the liver. Brunt et al classification of NAFLD severity categories span from Grade 0 (less than 5% fat) to Grade III (over 66%)[40]. The vast majority of our cohort (68.8%) had a grade 0, 27.8% had a grade I, and 1% had a grade II. This indicates that even a relatively low accumulation of fat in the liver is associated with changes in brain volume and cognition. Similarly, to the finding that NAFLD is related to lower intracranial volume[41], we found that higher hepatic fat % was associated with lower GM volume. A systematic review that included eleven observational studies (n=7978) showed that older adults with NAFLD had poor cognitive performance in global cognition, attention, and mental flexibility[42]. We speculate that the lack of associations between hepatic fat and cognition in our study is due to the younger age of IRAP participants and because NAFLD may lead to hampered cognition by additional pathways regardless of fat %.

Consistent with our finding of an association of higher VAT and SAT % with MFG and SFG volumes in middle-aged adults, higher VAT and SAT % were associated with lower cortical thickness[10] and smaller brain volumes, specifically in regions related to memory and cognitive function, in older adults[10,11]. Regarding cognition, high VAT was associated with reduced cognitive scores in a recent large study (n=9189), and specifically with lower memory, attention, and processing speed, in older adults in another study[9]. SAT was associated with worsening cognitive function after 7 years in men[12], while it was found to be protective among women, who had a lower risk of dementia[13]. In these studies, VAT quantification was done in L4-L5, while we quantified VAT and SAT by averaging 4 abdominal levels (L2-S1), which may better reflect the abdominal VAT and SAT distribution. The lack of association between VAT and SAT with cognition in our sample may be related to its relatively young age, as the deleterious effects of VAT and SAT, in contrast to pancreatic fat, may be cumulatively expressed with greater impact later in life. Although there was no difference in BMI between males and females – BMI was associated with SAT % mostly in females. These results are in adherence with previous publications, that showed that high BMI is associated with high hepatic fat[43], high pancreatic fat[44], and SAT layers[45], while VAT % did not correlate with BMI[45]. We also found that pancreatic fat was associated with hepatic fat primarily in males (Table S2), while hepatic fat was associated with VAT mainly in females, and with SAT regardless of sex. This suggests that hepatic and pancreatic fat are differentially associated with each other in males and females enhancing the need to address each fat depot separately.

Strengths of the study include a relatively large sample of individuals who underwent abdominal MRI, cognitive assessments, and brain imaging. MRI assessment of fat enabled accurate quantification of abdominal fat depots with MATLAB-based semiautomatic method[22]. We used a broad cognitive battery which allowed for the assessment of 4 cognitive domains in addition to global cognition. The study also had limitations, primarily the cross-sectional design that limits causal inferences. The sample size of participants with cognitive data (N=204) and with MRI data (N=142) are relatively small for firm conclusions. In addition, the IRAP sample is based on middle-aged offspring of AD patients and therefore it does not represent the population of middle-aged adults as a whole but rather those at high risk of developing AD. To more definitively establish the associations between fat accumulation, neurodegeneration, and cognitive decline, future studies must replicate these findings using

larger and more representative samples, incorporating longitudinal designs. Additionally, the study did not investigate potential mechanisms underlying the observed associations, such as menopausal status or treatment, inflammation, insulin resistance, and factors secreted from the different fat depots and other potential confounders such as daily exercise and dietary factors. These factors may contribute to fat accumulation and are related to brain health.

In summary, the current study provides valuable insight into the associations between different abdominal fat depots and brain health outcomes, after adjusting for a broad range of potential sociodemographic, clinical, and functional confounders. These findings underscore the importance of investigating the inter-relationships of fat depots, brain aging, and cognition in the context of sex differences. Further research is needed to confirm these findings and investigate the underlying mechanisms that may explain the observed associations, which may lead to sex-specific interventions for the promotion of brain health. Finally, our study provides new evidence pointing to the contribution of distinct fat depots to brain aging and suggests that global obesity measures such as BMI may fail to identify important links in the fat-brain-cognition pathway.

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Table 1

Demographic and clinical characteristics

	Whole sample				Brain MRI subsample			
Characteristics	Whole sample	Males (n=80)	Females (n=124)	t-test/ Chi-Square/ ANCOVA (p)	Whole sample	Males (n=60)	Females (n=82)	t-test/ Chi-Square/ ANCOVA (p)
Demographic								
Age years (SD)	59.44 (7.60)	58.99 (7.15)	59.73 (7.89)	-0.36 (0.72)	61.16 (7.27)	60.78 (6.79)	61.43 (7.63)	-0.53 (0.60)
Education years (SD)	16.88 (2.93)	17.01 (2.97)	16.80 (2.92)	0.49 (0.62)	17.16 (2.89)	17.39 (2.90)	16.98 (2.88)	0.84 (0.40)
Clinical								
BMI kg/m^2 (SD)	26.70 (4.64)	26.54 (4.28)	26.80 (4.87)	-0.39 (0.70)	25.95 (3.79)	25.81 (3.04)	26.04 (4.27)	-0.36 (0.36)
T2D n (%)	14 (6.9%)	8 (10%)	6 (4.8%)	0.73 (0.69)	4 (6.7%)	7 (4.9%)	3 (3.7%)	0.67 (0.41)
HbA1c % (SD)	5.51 (0.59)	5.49 (0.56)	5.52 (0.61)	-0.30 (0.77)	5.49 (0.59)	5.45 (0.51)	5.42 (0.44)	0.72 (0.24)
Cholesterol mg/dL (SD)	202.50 (36.73)	190.26 (33.29)	210.21 (36.83)	**-3.76 (<0.001)**	192.21 (36.01)	200.11 (36.34)	205.92 (35.70)	**-2.17 (0.02)**
Systolic blood pressure mmHg (SD)	127.90 (18.72)	133.40 (18.62)	124.35 (17.98)	**3.46 (<0.001)**	132.89 (16.56)	127.36 (18.10)	123.31 (18.20)	**3.22 (<0.001)**
Diastolic blood pressure mmHg (SD)	79.38 (10.61)	82.82 (10.26)	77.16 (10.27)	**3.85 (<0.001)**	82.88 (9.92)	78.97 (10.11)	76.11 (9.32)	**4.17 (<0.001)**
Smoking status No/Yes/Past n (%)	145/21/38 (71.1/10.3/18.6)	56/10/14 (70/12.5/17.5)	89/11/24 (71.8/8.9/19.4)	2.03 (0.16)	47/8/5 (78.3/13.3/8.3)	110/13/19 (77.5/9.2/13.4)	63/5/14 (76.8/6.1/17.1)	3.97 (0.14)
Regional abdominal fat								
Hepatic fat % (SD)	5.68 (5.67)	5.42 (5.88)	5.85 (5.55)	-0.52 (0.60)	4.89 (5.36)	4.89 (5.36)	5.34 (4.82)	-0.53 (0.60)
Pancreatic fat % (SD)	6.95 (5.98)	8.06 (6.66)	6.27 (5.44)	**1.99 (0.05)**	7.68 (5.90)	7.68 (5.90)	6.29 (4.93)	1.48 (0.14)
VAT % (SD)	33.59 (10.00)	41.34 (8.56)	28.65 (7.38)	**11.21 (<0.001)**	42.06 (7.96)	42.06 (7.96)	29.14 (7.08)	**10.19 (<0.001)**
SAT % (SD)	57.79 (9.68)	50.40 (8.02)	62.49 (7.46)	**-10.93 (<0.001)**	49.95 (7.68)	49.95 (7.68)	62.26 (7.16)	**-9.81 (<0.001)**
Cognitive domain								
Executive function Z (SD)	0.00 (0.78)	-0.02 (0.80)	0.02 (0.77)	-0.33 (0.74)	0.02 (0.80)	0.02 (0.80)	0.06 (0.83)	-0.28 (0.78)
Episodic memory Z (SD)	0.00 (0.86)	-0.21 (0.83)	0.13 (0.86)	**-2.78 (0.01)**	-0.17 (0.82)	-0.17 (0.82)	0.17 (0.83)	**-2.41 (0.02)**
Working memory Z (SD)	0.00 (0.77)	0.11 (0.79)	-0.07 (0.75)	1.60 (0.11)	0.19 (0.80)	0.19 (0.80)	-0.09 (0.70)	**2.17 (0.03)**
Language Z (SD)	0.00 (0.80)	0.01 (0.77)	-0.00 (0.82)	0.07 (0.95)	0.11 (0.67)	0.11 (0.67)	-0.00 (0.84)	0.86 (0.39)
Global cognition Z (SD)	0.00 (0.57)	-0.03 (0.60)	0.02 (0.56)	-0.58 (0.56)	0.04 (0.58)	0.04 (0.58)	0.04 (0.55)	0.02 (0.98)
Brain volumes								
TIV cm^3 (SD)					1433.63 (136.53)	1543.47 (94.58)	1353.26 (102.27)	**11.30 (<0.001)**
Total GM cm^3 (SD)					588.04 (48.81)	624.48 (33.57)	560.83 (39.90)	**4.06 (0.05)**

Hippocampus cm^3 (SD)		6.31 (0.56)	6.64 (0.50)	6.06 (0.48)	0.30 (0.59)
IFG cm^3 (SD)		13.17 (1.31)	13.86 (1.13)	12.66 (1.19)	0.42 (0.52)
MFG cm^3 (SD)		29.81 (3.24)	31.69 (2.76)	28.43 (2.86)	0.18 (0.67)
SFG cm^3 (SD)		23.92 (2.64)	25.63 (2.16)	22.67 (2.24)	3.27 (0.07)

T-test, Pearson Chi-Square, and ANCOVA for comparison between males and females. Abbreviations: MRI, magnetic resonance imaging; SD, standard deviation; BMI, body mass index; T2D, Type 2 diabetes; HbA1c, hemoglobin A1c; mg/dL, milligram/deciliter; mmHg, millimeters of mercury; VAT, visceral adipose tissue; SAT, subcutaneous adipose tissue; cm, centimeters; TIV, total intracranial volume; GM, Grey matter; IFG, inferior frontal gyrus; MFG, middle frontal gyrus; SFG, superior frontal gyrus.

Table 2

Associations of regional abdominal adiposity with BMI

Regional abdominal fat	Whole sample (n=204)		Males (n=80)		Females (n=124)	
	β	*p*	β	*p*	β	*p*
Hepatic fat %	**0.32**	**<0.001**	**0.29**	**0.02**	**0.35**	**<0.001**
Pancreatic fat %	**0.37**	**<0.001**	**0.38**	**<0.001**	**0.38**	**<0.001**
VAT %	-0.03	0.57	0.04	0.75	-0.05	0.58
SAT %	**0.15**	**0.01**	0.04	0.75	**0.23**	**0.01**

Linear regression for associations of regional abdominal adiposity with BMI males and females. Covariates: age, sex, education, and the time between assessments. Abbreviations: BMI, body mass index; VAT, visceral adipose tissue; SAT, subcutaneous adipose tissue.

Table 3

Associations of ectopic abdominal adiposity with cognitive functioning and brain volumes

		A				B			
		Hepatic fat %				Pancreatic fat %			
		Model 1		Model 2		Model 1		Model 2	
		β	p	β	p	β	p	β	p
Cognitive domain									
Episodic Memory	Entire sample	-0.03	0.73	0.01	0.95	-0.06	0.47	-0.07	0.37
	Males	-0.06	0.62	-0.03	0.80	**-0.28**	**0.03**	-0.26	0.07
	Female	-0.00	0.97	0.02	0.80	0.07	0.48	0.01	0.93
	Interaction [a]	0.08	0.75	0.10	0.72	0.51	**0.05**	0.38	0.17
Working memory	Entire sample	-0.02	0.84	-0.01	0.94	-0.07	0.38	-0.06	0.49
	Males	0.09	0.47	0.02	0.87	-0.07	0.61	-0.03	0.84
	Female	-0.09	0.35	-0.06	0.56	-0.08	0.43	-0.12	0.29
	Interaction [a]	-0.31	0.25	-0.15	0.62	-0.05	0.85	-0.17	0.56
Executive function	Entire sample	-0.06	0.42	-0.01	0.89	-0.11	0.15	-0.10	0.23
	Males	-0.03	0.81	0.05	0.72	**-0.27**	**0.03**	**-0.32**	**0.02**
	Female	-0.08	0.40	-0.01	0.92	0.02	0.87	0.00	0.97
	Interaction [a]	-0.09	0.74	-0.10	0.73	0.44	**0.08**	0.50	**0.06**
Language	Entire sample	-0.05	0.57	-0.04	0.69	0.07	0.44	0.06	0.56
	Males	0.02	0.86	0.06	0.65	-0.18	0.16	**-0.33**	**0.03**
	Female	0.09	0.37	0.14	0.17	0.01	0.93	-0.01	0.90
	Interaction [a]	0.13	0.63	0.17	0.55	0.27	0.31	0.41	0.17
Global cognition	Entire sample	-0.01	0.86	0.03	0.67	-0.11	0.17	-0.11	0.17
	Males	0.01	0.95	0.03	0.81	-0.27	**0.03**	**-0.33**	**0.02**
	Female	-0.03	0.78	0.04	0.68	0.01	0.93	-0.04	0.70
	Interaction [a]	-0.06	0.82	0.02	0.95	0.42	0.10	0.41	0.12
Brain volumes									
GM	Entire sample	**-0.13**	**0.00**	-0.08	0.15	0.06	0.26	0.09	0.08
	Males	**-0.23**	**0.02**	-0.08	0.56	0.09	0.42	0.14	0.18
	Female	**-0.18**	**0.02**	-0.15	0.10	0.07	0.38	0.09	0.32
	Interaction [a]	0.00	0.98	-0.12	0.55	0.03	0.86	-0.02	0.92
Hippocampus	Entire sample	-0.07	0.24	-0.13	0.08	-0.12	0.09	-0.11	0.14
	Males	-0.06	0.58	-0.18	0.24	0.01	0.91	0.15	0.26
	Female	-0.13	0.19	-0.22	0.07	**-0.28**	**0.01**	**-0.25**	**0.03**
	Interaction [a]	-0.11	0.62	-0.07	0.79	-0.45	**0.05**	-0.57	**0.02**
IFG	Entire sample	-0.07	0.26	-0.03	0.69	-0.07	0.31	-0.02	0.75
	Males	-0.17	0.15	-0.20	0.19	**-0.28**	**0.02**	-0.18	0.15
	Female	-0.02	0.80	0.06	0.63	0.10	0.33	0.09	0.43
	Interaction [a]	0.20	0.38	0.38	0.19	0.52	**0.02**	0.38	0.13
MFG	Entire sample	-0.04	0.49	0.01	0.94	0.03	0.66	0.08	0.28
	Males	-0.13	0.29	-0.01	0.94	0.03	0.85	0.17	0.27
	Female	-0.01	0.95	0.02	0.85	0.05	0.66	0.06	0.62

	Interaction [a]	0.17	0.45	0.05	0.87	0.05	0.85	-0.12	0.66
SFG	Entire sample	-0.02	0.74	0.03	0.72	-0.00	0.96	0.01	0.92
	Males	0.03	0.77	0.13	0.47	-0.12	0.38	-0.14	0.40
	Female	-0.08	0.42	-0.01	0.91	0.10	0.37	0.09	0.45
	Interaction [a]	-0.17	0.44	-0.19	0.52	0.30	0.21	0.30	0.25

Model 1 covariates: age, sex, education, the time between assessments, BMI, and TIV (for brain volumes only).

Model 2 covariates: age, sex, education, the time between assessments, BMI, diastolic blood pressure, systolic blood pressure, total cholesterol, HbA1c, and TIV (for brain volumes only).

[a] Interactions between regional fat and sex on cognitive function/ brain volumes.

Significant values are marked in bold.

Abbreviations: GM, Grey matter; IFG, inferior frontal gyrus; MFG, middle frontal gyrus; SFG, superior frontal gyrus.

Table 4

Associations of VAT and SAT % with cognitive functioning and brain volumes

		A				B			
		VAT %				SAT %			
		Model 1		Model 2		Model 1		Model 2	
		β	p	β	p	β	p	β	p
Cognitive domain									
Episodic Memory	Entire sample	-0.07	0.42	-0.00	0.97	0.09	0.29	0.00	0.98
	Males	-0.15	0.16	-0.05	0.64	0.20	0.06	0.07	0.52
	Female	-0.00	0.96	0.02	0.88	-0.01	0.90	-0.06	0.58
	Interaction [a]	0.24	0.36	0.12	0.66	-0.97	0.18	-0.65	0.40
Working memory	Entire sample	0.12	0.18	0.10	0.28	-0.06	0.49	-0.04	0.68
	Males	0.12	0.29	0.08	0.50	-0.05	0.66	-0.01	0.94
	Female	0.05	0.61	0.10	0.35	-0.03	0.80	-0.07	0.56
	Interaction [a]	-0.13	0.64	0.05	0.87	0.13	0.87	-0.30	0.72
Executive function	Entire sample	-0.04	0.62	0.01	0.89	0.06	0.52	-0.01	0.91
	Males	0.04	0.75	-0.01	0.96	-0.04	0.71	-0.01	0.93
	Female	-0.07	0.40	0.02	0.85	0.10	0.28	0.00	0.97
	Interaction [a]	-0.21	0.43	0.05	0.87	0.70	0.32	0.07	0.93
Language	Entire sample	-0.05	0.57	-0.04	0.69	0.07	0.44	0.06	0.56
	Males	-0.02	0.86	-0.09	0.42	-0.00	0.97	0.08	0.50
	Female	-0.06	0.52	0.00	0.97	0.10	0.31	0.05	0.69
	Interaction [a]	-0.09	0.74	0.16	0.60	0.55	0.46	-0.10	0.91
Global cognition	Entire sample	-0.02	0.84	0.03	0.77	0.06	0.51	0.00	0.96
	Males	-0.01	0.96	-0.02	0.82	0.04	0.73	0.05	0.68
	Female	-0.03	0.71	0.05	0.63	0.06	0.54	-0.03	0.79
	Interaction [a]	-0.05	0.84	0.14	0.62	0.11	0.88	-0.36	0.64
Brain volumes									
GM	Entire sample	0.04	0.55	0.11	0.07	-0.02	0.78	-0.10	0.10
	Males	0.06	0.56	0.08	0.43	-0.06	0.52	-0.11	0.26
	Female	0.02	0.80	0.10	0.24	0.02	0.75	-0.06	0.54
	Interaction [a]	-0.04	0.83	0.07	0.71	0.32	0.51	0.14	0.79
Hippo campus	Entire sample	-0.04	0.63	-0.07	0.40	0.07	0.40	0.12	0.15
	Males	-0.03	0.76	-0.09	0.45	0.07	0.51	0.16	0.17
	Female	0.01	0.95	-0.04	0.74	0.00	0.97	0.04	0.73
	Interaction [a]	0.06	0.79	0.07	0.78	-0.29	0.65	-0.49	0.50

IFG	Entire sample	0.023	0.76	0.08	0.36	-0.02	0.85	-0.08	0.35
	Males	0.02	0.86	0.01	0.94	-0.04	0.74	-0.05	0.68
	Female	0.05	0.63	0.15	0.17	-0.01	0.93	-0.11	0.33
	Interaction [a]	0.05	0.83	0.26	0.34	0.12	0.86	-0.32	0.67
MFG	Entire sample	0.14	0.08	**0.21**	**0.02**	-0.11	0.19	**-0.18**	**0.04**
	Males	0.22	0.07	**0.29**	**0.03**	-0.18	0.12	**-0.27**	**0.03**
	Female	0.08	0.38	0.11	0.34	-0.04	0.68	-0.07	0.56
	Interaction [a]	-0.18	0.45	-0.27	0.33	0.59	0.38	0.89	0.25
SFG	Entire sample	0.01	0.87	0.07	0.44	0.00	0.96	-0.04	0.61
	Males	0.23	0.05	**0.31**	**0.02**	-0.13	0.27	-0.20	0.14
	Female	-0.16	0.09	-0.17	0.14	0.13	0.21	0.12	0.12
	Interaction [a]	-0.61	**0.01**	**-0.74**	**0.01**	1.11	**0.09**	1.38	**0.07**

Model 1 covariates: age, sex, education, the time between assessments, BMI, and TIV (for brain volumes only).

Model 2 covariates: age, sex, education, the time between assessments, BMI, diastolic blood pressure, systolic blood pressure, total cholesterol, HbA1c, and TIV (for brain volumes only).

[a] Interactions between regional fat and sex on cognitive function/ brain volumes.

Significant values are marked in bold.

Abbreviations: VAT, visceral adipose tissue; SAT, subcutaneous adipose tissue; GM, Grey matter; IFG, inferior frontal gyrus; MFG, middle frontal gyrus; SFG, superior frontal gyrus.

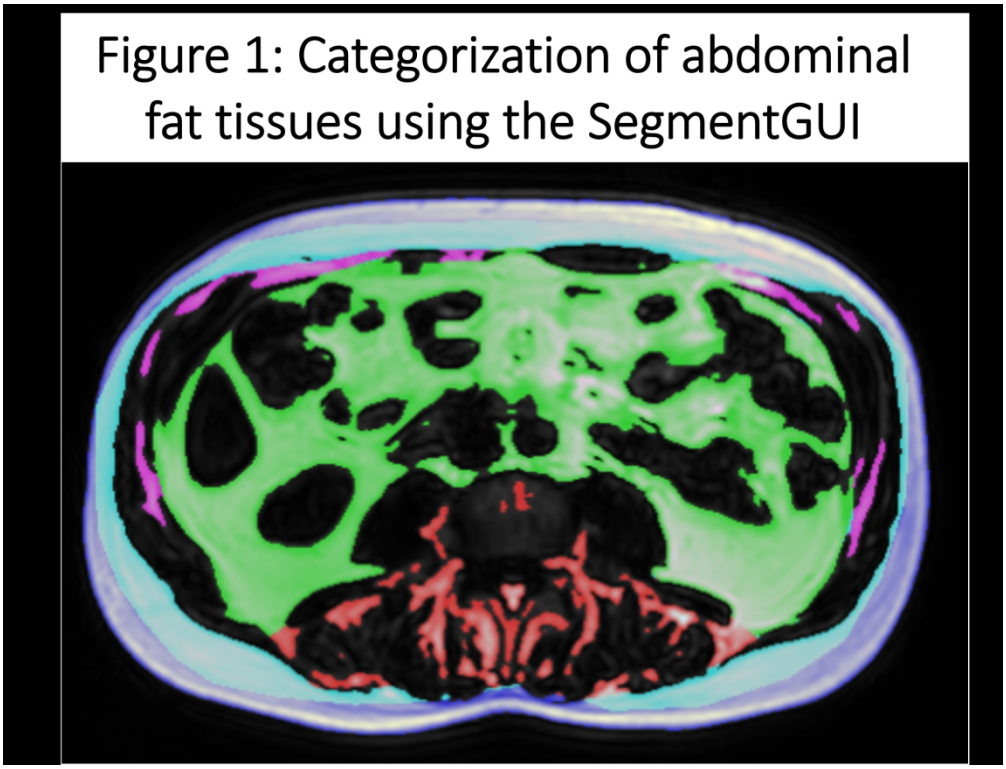

Figure 1: Representative image of SegmentGUI. Abdominal fat tissues were manually categorized into color-coded groups, which included SAT (purple and light blue), VAT (green), peri-muscular fat (pink), and non-classified fat (red).

453x344mm (130 x 130 DPI)

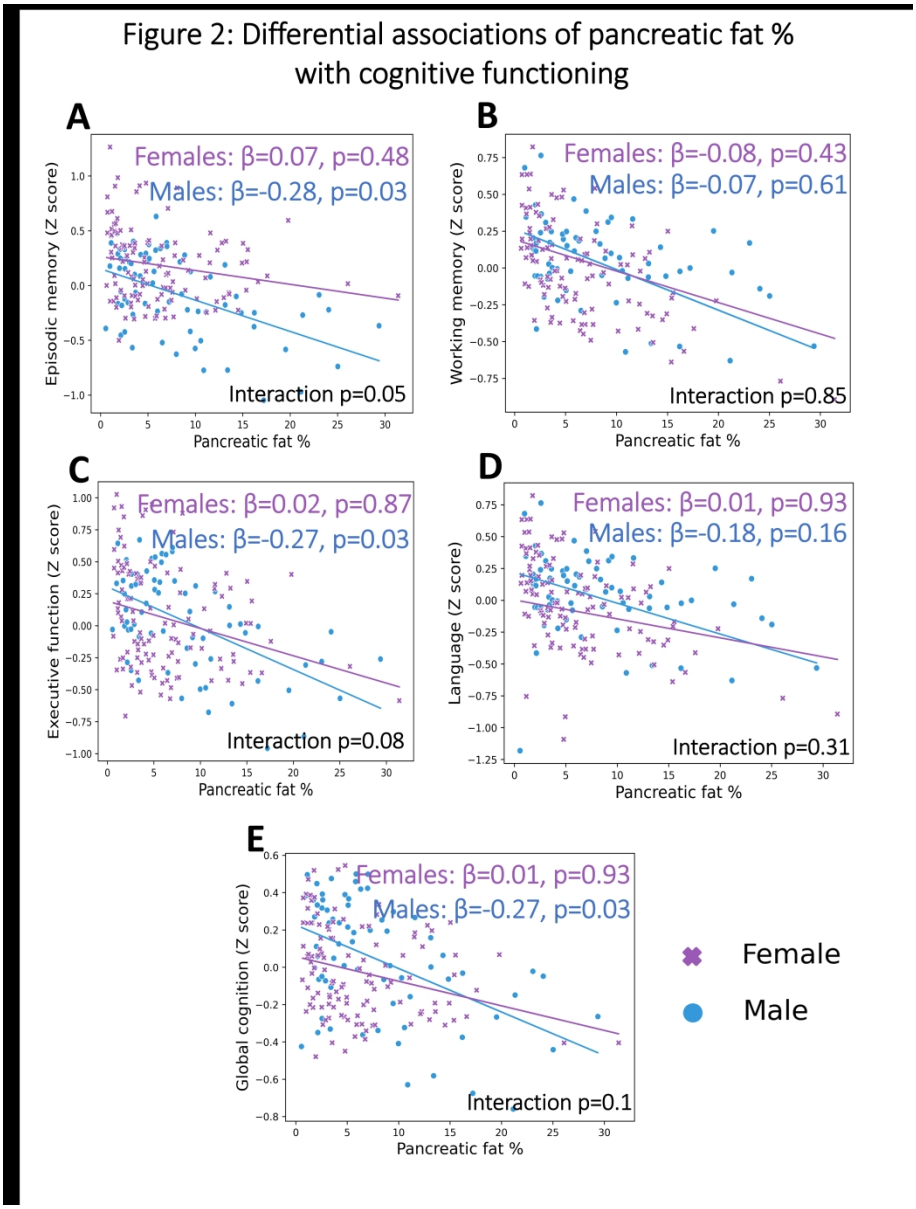

Figure 2: Scatterplots depicting different associations of pancreatic fat % with cognitive functioning in males (blue) and females (purple). Each line is representative of the results of the linear regression of model 1. Model 1 covariates: age, sex, education, the time between assessments, and BMI. The p-value of the interaction between pancreatic fat and sex (male/female) on cognitive functioning is presented.

798x1052mm (130 x 130 DPI)

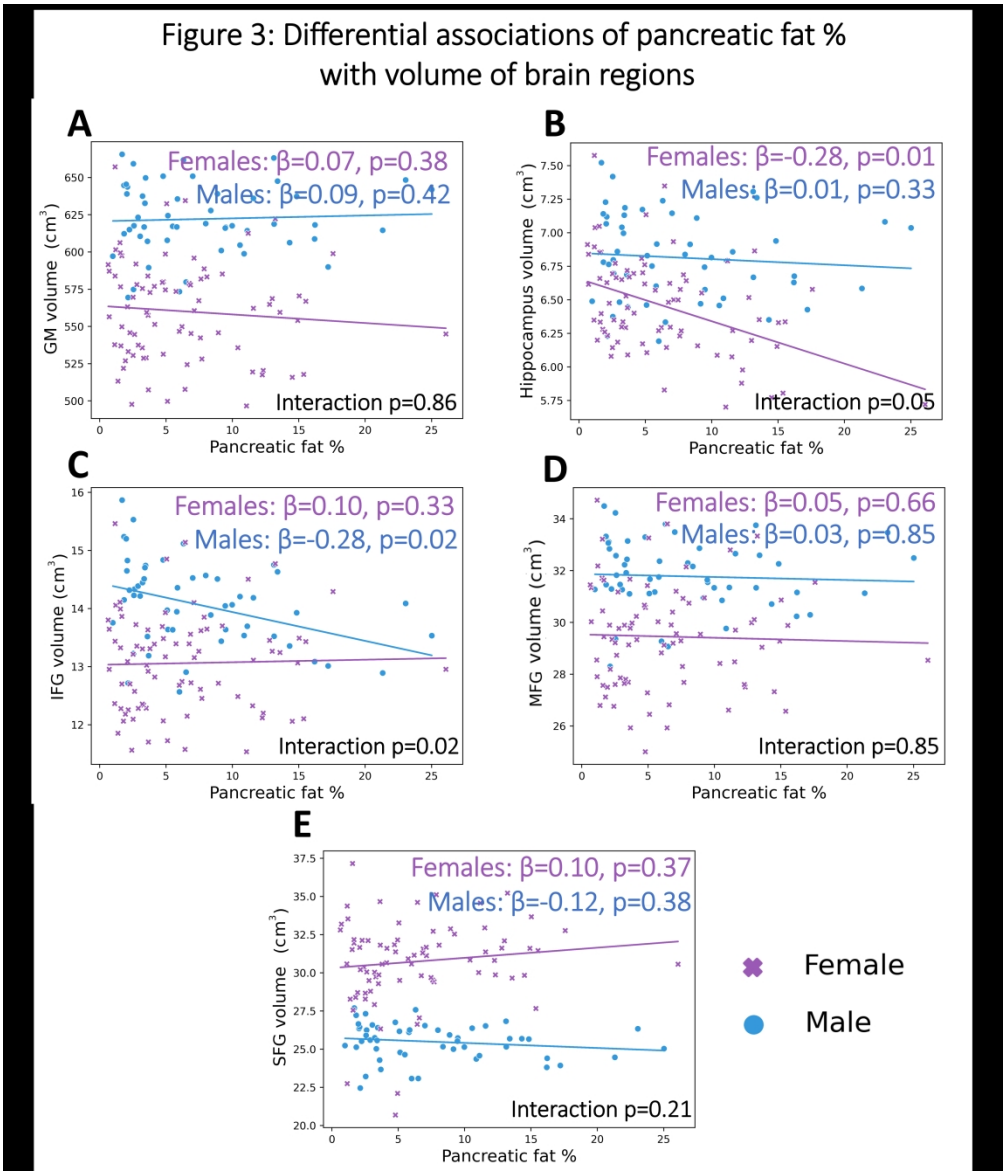

Figure 3: Scatterplots depicting different associations of pancreatic fat % with volume of brain regions in males (blue) and females (purple). Each line is representative of the results of the linear regression of model 1. Model 1 covariates: age, sex, education, the time between assessments, BMI, and TIV. The p-value of the interaction between pancreatic fat and sex (male/female) on AD-related brain volumes is presented. Abbreviations: GM, Grey matter; cm, centimeters; IFG, inferior frontal gyrus; MFG, middle frontal gyrus; SFG, superior frontal gyrus.

860x1004mm (130 x 130 DPI)

Figure 4: Differential associations of VAT and SAT % with SFG volume

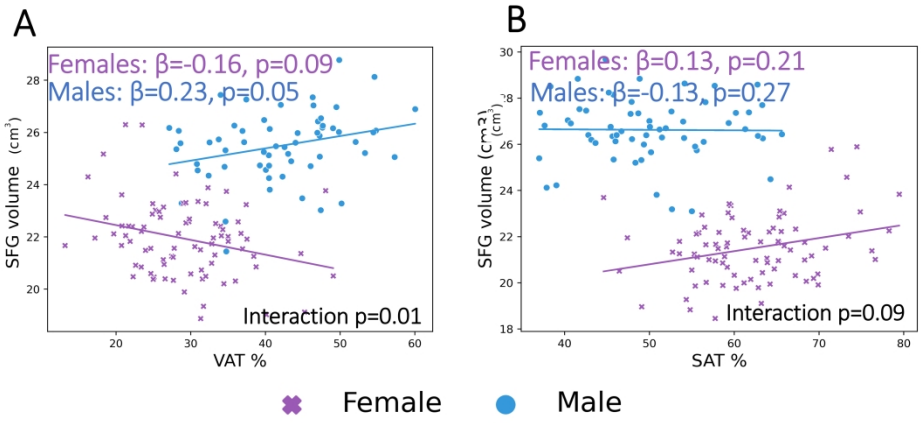

Figure 4: Scatterplots depicting different associations of VAT and SAT % with SFG volume in males (blue) and females (purple). Each line is representative of the results of the linear regression of model 1. The p-value of the interaction between pancreatic fat and sex (male/female) on cognitive functioning is presented. Model 1 covariates: age, sex, education, the time between assessments, BMI, and TIV. Abbreviations: SFG, superior frontal gyrus; cm, centimeters.

871x446mm (130 x 130 DPI)

Table S1

Associations of abdominal adiposity with cognitive functioning and brain volumes – adjusting for demographics and T2D status

		Hepatic fat %		Pancreatic fat %		VAT %		SAT %	
Cognitive domain									
		β	p	β	p	β	p	β	p
Episodic Memory	Entire sample	-0.01	0.88	-0.06	0.45	-0.07	0.44	0.09	0.31
	Males	-0.01	0.93	**-0.29**	**0.02***	-0.16	0.14	0.21	0.05
	Female	-0.01	0.96	0.07	0.48	-0.00	0.97	-0.01	0.89
	Interaction	0.01	0.98	0.53	**0.05+**	0.26	0.33	-1.02	0.16
Working memory	Entire sample	-0.00	0.98	-0.07	0.36	0.13	0.17	-0.07	0.47
	Males	0.13	0.29	-0.07	0.58	0.12	0.31	-0.05	0.68
	Female	-0.10	0.35	-0.08	0.43	0.05	0.63	-0.02	0.82
	Interaction	-0.39	0.15	-0.04	0.88	-0.13	0.65	0.12	0.88
Executive function	Entire sample	-0.04	0.59	-0.11	0.14	-0.04	0.64	0.053	0.54
	Males	0.05	0.69	**-0.28**	**0.02***	**-0.40**	**0.00***	-0.03	0.79
	Female	-0.08	0.39	0.02	0.87	-0.08	0.34	0.11	0.23
	Interaction	-0.22	0.39	0.46	**0.06+**	-0.21	0.43	0.70	0.32
Language	Entire sample	-0.04	0.64	0.06	0.52	-0.04	0.64	0.06	0.52
	Males	0.14	0.20	-0.21	0.07	-0.04	0.69	0.02	0.87
	Female	0.10	0.31	0.01	0.96	-0.05	0.61	0.09	0.37
	Interaction	-0.04	0.87	0.30	0.24	-0.03	0.92	0.40	0.59
Global cognition	Entire sample	0.02	0.78	-0.11	0.13	-0.01	0.90	0.05	0.56
	Males	0.10	0.34	**-0.29**	**0.01***	-0.02	0.84	0.05	0.61
	Female	-0.02	0.80	0.01	0.94	-0.03	0.72	0.06	0.55
	Interaction	-0.22	0.38	0.45	**0.07+**	-0.02	0.93	0.03	0.96
Brain volumes									
GM	Entire sample	**-0.16**	**<0.00***	0.06	0.25	0.04	0.56	-0.02	0.78
	Males	**-0.25**	**0.03***	0.08	0.45	0.03	0.77	-0.03	0.73
	Female	**-0.20**	**0.01***	0.08	0.34	-0.00	0.99	0.04	0.62
	Interaction	-0.01	0.94	0.05	0.78	-0.04	0.84	0.29	0.55
Hippocampus	Entire sample	-0.07	0.29	-0.12	0.09	-0.04	0.64	0.07	0.40
	Males	0.11	0.42	-0.01	0.96	-0.07	0.50	0.11	0.29
	Female	-0.16	0.12	**-0.27**	**0.01***	-0.01	0.88	0.02	0.86
	Interaction	-0.39	0.11	-0.42	**0.06+**	0.09	0.71	-0.41	0.53

IFG	Entire sample	-0.09	0.21	-0.07	0.32	0.03	0.76	-0.02	0.85
	Males	-0.22	0.13	**-0.28**	**0.02***	0.01	0.92	-0.03	0.80
	Female	-0.03	0.74	0.10	0.32	0.04	0.68	-0.00	0.98
	Interaction	0.25	0.32	0.53	**0.02+**	0.05	0.83	0.11	0.87
MFG	Entire sample	-0.07	0.34	0.03	0.64	0.14	0.08	-0.11	0.19
	Males	-0.11	0.49	0.02	0.89	0.20	0.11	-0.16	0.18
	Female	-0.03	0.77	0.05	0.63	0.06	0.52	-0.02	0.80
	Interaction	0.10	0.70	0.06	0.80	-0.18	0.46	0.56	0.41
SFG	Entire sample	-0.00	0.99	-0.00	0.96	0.01	0.86	0.01	0.94
	Males	0.06	0.68	-0.12	0.38	0.22	0.07	-0.12	0.32
	Female	-0.06	0.55	0.10	0.37	-0.15	0.13	0.11	0.26
	Interaction	-0.18	0.48	0.29	0.22	-0.58	**0.02+**	1.02	0.13

Covariates: age, sex, education, time between assessments, BMI, T2D status (yes/no), and TIV (for brain volumes only).

[a] Interactions between regional fat and sex on cognitive function/ brain volumes.

* $p < 0.05$; + $p<0.1$ for interaction.

Abbreviations: T2D, type 2 diabetes; VAT, visceral adipose tissue; SAT, subcutaneous adipose tissue; GM, Grey matter; IFG, inferior frontal gyrus; MFG, middle frontal gyrus; SFG, superior frontal gyrus.

Table S2

Associations of regional fat depots

		Pancreatic fat		**Hepatic fat**		**VAT**	
		β	**p**	**β**	**p**	**β**	**p**
Hepatic fat	**Whole sample**	**0.18**	**0.01**				
	Males	**0.27**	**0.02**				
	Females	0.12	0.20				
VAT	**Whole sample**	**0.21**	**0.01**	**-0.16**	**0.03**		
	Males	0.18	0.15	0.16	0.17		
	Females	0.13	0.17	**0.37**	**<0.00**		

SAT	Whole sample	-0.19 0.01	0.19	0.01	-0.93 <0.00
	Males	-0.18 0.15	-0.21	0.07	-0.90 <0.00
	Females	-0.09 0.33	-0.25	0.01	-0.88 <0.00

Linear regression of regional fat depots.

Abbreviations: VAT, visceral adipose tissue; SAT, subcutaneous adipose tissue.