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Amygdalar activity predicts future incident diabetes independently of adiposity

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ABSTRACT

While it is established that psychosocial stress increases the risk of developing diabetes mellitus (DM), two key knowledge gaps remain: 1) the neurobiological mechanisms that are involved in mediating that risk, and 2) the role, if any, that adiposity plays in that mechanism. We tested the hypotheses that: 1) metabolic activity in the amygdala (AmygA), a key center involved in the neurobiological response to stress, associates with subsequent DM risk, and 2) this association is independent of adiposity. AmygA and adipose tissue volumes were measured, and serial blood assessments for DM were obtained in 232 subjects who underwent combined ^{18}F-fluorodeoxyglucose positron emission tomography/computed tomography (^{18}F-FDG-PET/CT) imaging. Higher baseline AmygA predicted subsequent, new-onset DM, independently of adiposity and other DM risk factors. Furthermore, higher adiposity only increased DM risk in the presence of higher AmygA. In a separate cross-sectional cohort, higher AmygA associated with higher insulin resistance. Accordingly, the current study shows, for the first time, that activity in a stress-responsive neural region predicts the onset of DM. Further, we observed that this neurobiological activity acts independently of, but also synergistically with adiposity to increase DM risk. These findings suggest novel therapeutic targets to help manage and possibly prevent DM.

1. Introduction

Diabetes mellitus (DM) represents a rapidly growing threat to global health (Mokdad et al., 2003). The development of DM is closely associated with obesity and, more potently, with excess visceral adipose tissue (VAT) (Neeland et al., 2012). Yet, most obese individuals do not develop DM, underscoring the fact that additional variables contribute to the development of diabetes (Rosen and Spiegelman, 2006). Psychosocial stress represents such a factor (Pouwer et al., 2010). Epidemiologic evidence suggests that stress adversely impacts glycemic control among individuals with pre-existing DM (Chida and Hamer, 2008) and contributes to the development of DM (Kumari et al., 2004; Mooy et al., 2000). However, the mechanistic pathway that links stress to DM remains incompletely defined.

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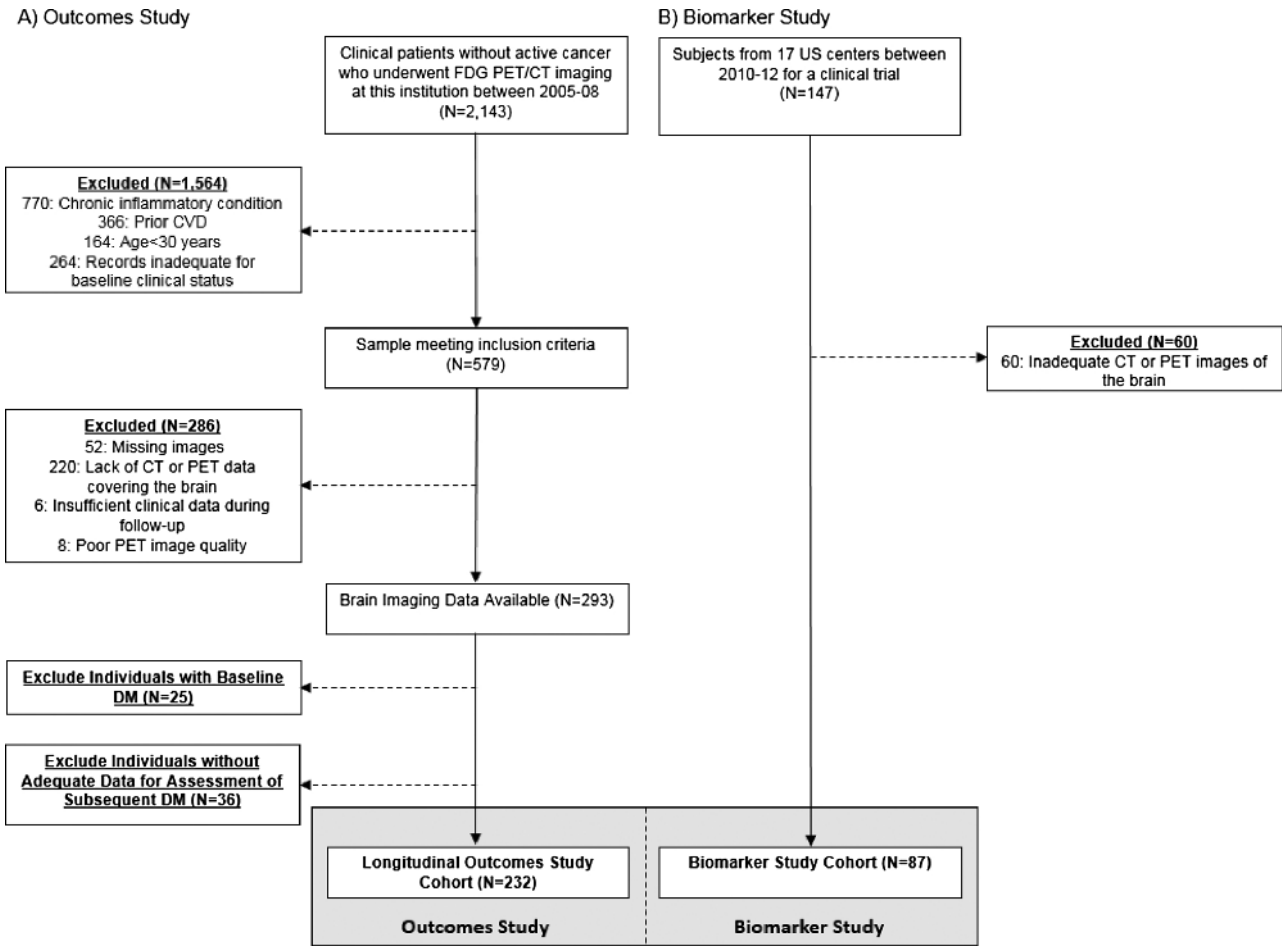

Fig. 1. (A) Outcomes study subject selection. (B) Biomarker study subject selection.
Abbreviations: CVD: cardiovascular disease, CT: computed tomography, DM: diabetes mellitus, FDG: fluorodeoxyglucose, LDL: low density lipoprotein, PET: positron emission tomography.

Stress associates with increased adiposity (notably VAT), in part due to its association with adverse health behaviors, such as excess caloric intake and physical inactivity (Adam and Epel, 2007; Kouvonen et al., 2005). Since increased VAT is a potent risk factor for DM, it would be appealing to simply rely on measurements of VAT to gain insights into the risk of DM in the context of stress. However, prior work raised doubts about the importance of adiposity in mediating the risk of developing DM from stress (Mooy et al., 2000). In that cross-sectional study, although life stressors were associated with DM, adjusting for waist-to-hip ratio only marginally attenuated that association. Moreover, radiographic measures of adiposity (e.g., VAT) were not available. Accordingly, it remains unclear to what degree adiposity mediates the relationship between stress and subsequent DM.

Another key question, regarding the association between stress and DM, is which regional brain areas participate in the pathobiological mechanism. Translation of external stressors to their physiological consequences may involve activation of the brain's salience network, an ensemble of interconnected structures involved in complex functions such as cognition and emotion, among which the amygdala is an important component (Wang et al., 2010). Advanced imaging tools allow objective assessment of signals in brain regions (including the amygdala) that are known to be activated by psychosocial stress and stress conditions. Resting amygdalar metabolic activity (AmygA) can be measured using ^{18}F-fluorodeoxyglucose positron emission tomography/computed tomography (^{18}F-FDG-PET/CT) (Tawakol et al., 2017). The AmygA signal is reproducible, as indicated by an observed median change of only 2% in a clinically stable population over a three-month period (Hammad et al., 2017). AmygA associates with anxious temperament in animal models (Oler et al., 2010) and perceived stress in humans (Tawakol et al., 2017), and it is upregulated in chronic stress conditions (e.g., post-traumatic stress disorder, anxiety) (Bremner et al., 2005; Whalen et al., 2002). This signal may also provide important insights into the pathobiological consequences of stress, in that increased AmygA has previously been shown to robustly predict incident cardiovascular disease events in humans (Tawakol et al., 2017). Therefore, of the several brain regions potentially involved in the neurobiological response to stress, we prospectively hypothesized that the amygdala plays an important role in the mechanism linking chronic stress to DM.

^{18}F-FDG-PET/CT imaging is uniquely suited for investigating the relationship between the neurobiological response to stress and metabolic disease, since it enables simultaneous measurement of regional brain metabolic activity, using ^{18}F-FDG-PET, and volumetric measures of adipose tissues (e.g., VAT), using CT (Figueroa et al., 2016). Accordingly, we employed these imaging techniques and assessed for the development of new-onset type 2 DM to determine whether: 1) increased AmygA associates with subsequent new-onset DM and 2) this association is independent of adiposity.

2. Materials and methods

2.1. Overview

The study findings derive from two separate cohorts: 1) a

retrospective, longitudinal **outcomes study**, which evaluated the relationship between AmygA, adipose tissue volumes, and incident type 2 DM, and 2) a cross-sectional **biomarker study**, which evaluated the relationship between AmygA and biomarkers related to metabolism and inflammation. This study protocol was approved by Partners Human Research Committee/Institutional Review Board and was completed in agreement with the Declaration of Helsinki.

2.2. Study sample

Outcomes study subjects (N = 232) were identified from 2143 patients who had undergone clinical ^{18}F-FDG-PET/CT imaging (mostly for cancer screening) at the Massachusetts General Hospital (Boston, MA, USA) from 2005 to 2008 (Fig. 1A). Inclusion criteria were: 1) age > 30 years, 2) absence of known cardiovascular, inflammatory, or autoimmune disease at the time of imaging, 3) absence of prior malignancy or remission from cancer for at least one year prior to imaging and throughout follow-up, and 4) availability of at least three clinical notes in the medical records (spanning ≥ one year). This study's cohort represents a subset of the 293 subjects who participated in a prior study of AmygA and cardiovascular events (Tawakol et al., 2017). Of those, we excluded 25 individuals who had DM at baseline and 36 individuals who lacked adequate data for adjudication of subsequent type 2 DM. Accordingly, 232 individuals were included in the current analysis to test the relationship between AmygA and subsequent type 2 DM.

Biomarker study subjects (N = 87) were derived from a study of 147 individuals who participated in an ^{18}F-FDG-PET/CT imaging trial from 2010 to 2012 at seventeen United States centers (www.clinicaltrials.gov, NCT01258907) (Fig. 1B). Only the baseline cross-sectional data from that clinical trial are included in this study; that trial tested the impact of a drug among individuals with known or at high risk for cardiovascular disease and/or metabolic disease. Eligible subjects were between 35 and 80 years old with at least one of the following: 1) stable clinically or objectively diagnosed atherosclerotic disease, or 2) clinically diagnosed type 2 DM by American Diabetes Association (ADA) criteria (American Diabetes Association, 2016). Other inclusion and exclusion criteria have been previously reported (Lehrer-Graiwer et al., 2015). All trial subjects who had the brain within the imaging field of view were included in the current study.

2.3. Measurements

2.3.1. ^{18}F-FDG-PET/CT imaging protocol

^{18}F-FDG was administered intravenously at a dose of ~370 MBq following an overnight fast. Whole-body imaging was performed approximately one hour later using an integrated scanner (e.g., Biograph 64 Siemens Healthcare, Erlangen, Germany). Non-gated, non–contrast-enhanced CT was performed for attenuation correction. The imaging parameters are in accordance with the specifications of the Society of Nuclear Medicine Procedure Guideline for FDG PET Brain Imaging (Waxman et al., 2009).

2.3.2. Measurement of regional brain ^{18}F-FDG uptake

Resting AmygA was measured as previously described (Tawakol et al., 2017) by one blinded radiologist using a dedicated offline workstation (Leonardo TrueD, Siemens Healthcare, Forchheim, Germany) to fuse PET and CT datasets. The analysis leveraged approaches that have been used to evaluate the relationship of AmygA to temperament (Oler et al., 2010), stress-related disorders (Bremner et al., 2005; Whalen et al., 2002), and perceived stress (Tawakol et al., 2017). The amygdala, part of the limbic system located dorso-medially in the temporal lobe that forms the ventral superior and medial walls of the inferior horn of the lateral ventricle, was localized on the CT images by anatomic landmarks, as previously described (Tawakol et al., 2017). ^{18}F-FDG uptake was determined by placing circular (approximately 15 mm radius) regions of interest (ROIs) in the right and left amygdalae and measuring the mean tracer accumulation, quantified by the standardized uptake value (SUV), in each ROI. For the pre-specified endpoints, AmygA was defined as the mean amygdalar SUV corrected for background activity (i.e., mean temporal lobe SUV) (Britz-Cunningham et al., 2008). We pre-specified that AmygA was to be assessed both as a continuous variable and as a dichotomous variable (by median value). In order to evaluate the effect of amygdalar laterality and correction for alternative background tissues, post-hoc assessments of AmygA were performed, wherein the right and left amygdalae were assessed separately, cerebellar background activity was used to correct for background activity (Britz-Cunningham et al., 2008), and AmygA was divided into tertiles. Cerebral and cerebellar tissues were chosen for background correction because of their high but stable steady-state glucose metabolism.

2.3.3. Measurement of adipose tissue volumes

Adipose tissue volumes were measured, using the same offline imaging workstation, by a blinded investigator from simultaneously acquired CT images. Adipose tissue was identified using a threshold between -195 and -45 Hounsfield units. The abdominal muscular wall was used as a boundary to separate VAT and subcutaneous adipose tissue (SAT). The volumes were measured at the level of the umbilicus and expressed as cubic centimeters (cm^3) (Maurovich-Horvat et al., 2007).

2.3.4. Assessment of glucose homeostasis and determination of incident diabetes

The development of subsequent incident type 2 DM was determined using available clinical records by an investigator who was blinded to imaging data. Because the longitudinal outcomes study was retrospective, the frequency of subject follow-up and testing was based on individual clinician practices. Available fasting glucose (FBG) and glycohemoglobin (HbA1c) levels were assessed for two years before and up to five years after baseline imaging. Type 2 DM was adjudicated upon identification of a primary and a confirmatory measure of abnormal glucose homeostasis [i.e., two discrete measurements of either HbA1c ≥ 6.5% (48 mmol/mol) or FBG ≥ 126 mg/dL] in accordance with ADA criteria (American Diabetes Association., 2016). Similarly, all individuals who did not develop DM were evaluated for pre-diabetes at the two and five-year endpoints using ADA criteria [i.e., one measurement of HbA1c 5.7–6.4% (39–46 mmol/mol) or FBG 100–125 mg/dL] (American Diabetes Association., 2016). The onset of incident type 2 DM was defined as the date of the initial measure of abnormal glycemia within two years of index imaging to maximize the number of subjects with complete follow-up. Secondary analyses were performed for presence of DM at one, three or five years after index imaging.

2.3.5. Biomarker measurements

For the biomarker study, assays were performed using the HumanMAP1.6 multiplex panel from Rules Based Medicine. In addition, interleukin (IL)-6 and tissue necrosis factor (TNF) alpha immunoassays were performed using Quantikine R&D High Sensitivity ELISA, and IL-10 immunoassays were performed using kits from MSA at Pacific Biomarkers. These assays were conducted at a central core laboratory.

2.4. Statistical analyses

Statistical analyses were performed using SPSS (IBM Corp, Version 24). Continuous variables are given as mean ± standard deviation (SD) or median ± interquartile range (IQR) when not normally distributed. Associations were evaluated using Pearson and Spearman correlations for normally and non-normally distributed variables, respectively. Cox proportional hazards regression was used to calculate hazard ratios (HRs) and 95% confidence intervals (CIs). Kaplan-Meier estimates of DM-free survival were assessed to compare event-free

Table 1
Baseline characteristics of outcomes study subjects.

Characteristics	Full Cohort (N = 232)	Subgroups by Subsequent Development of Type 2 DM over 5 Years		
		No DM (N = 202)	DM (N = 30)	p-value (DM vs. No DM)
Age (years), median (IQR)	55.0 (44.0, 64.0)	53.8 (43.0, 63.0)	57.5 (47.5, 64.8)	0.16
Male, N (%)	98 (42.2)	79 (39)	19 (63)	**0.02**
Caucasian, N (%)	207 (89.2)	180 (89)	27 (90)	1.00
Current smoker, N (%)	19 (8.2)	15 (7)	4 (13)	0.28
Hypertension, N (%)	71 (30.6)	62 (31)	9 (30)	1.00
Hyperlipidemia, N (%)	60 (25.9)	51 (25)	9 (30)	0.66
Total cholesterol (mg/dL), Mean (SD)	194 (47)	195 (46)	189 (48)	0.63
Baseline Blood Glucose (mg/dL), median (IQR)	95 (88, 104)	94 (88, 103)	102 (94, 119)	**0.01**
LDL (mg/dL), Mean (SD)	113 (38)	114 (38)	109 (40)	0.62
Statin therapy, N (%)	39 (17)	31 (15)	8 (27)	0.12
FRS, Mean (SD)	5.3 (6.2)	5.1 (6.3)	6.7 (5.1)	0.30
BMI (kg/m^2), median (IQR)	26.4 (23.3, 30.7)	27.0 (22.8, 30.7)	28.6 (25.6, 31.6)	**0.04**
Visceral Adipose Tissue Volume (cm^3), median (IQR)	47.4 (26.3, 72.5)	46.8 (26.2, 68.1)	69.8 (26.9, 103.0)	**0.03**
History of cancer, N (%)	205 (88)	177 (88)	28 (93)	0.54
Prior chemotherapy, N (%)	191 (82)	164 (81)	27 (90)	0.31
History of Depression, N (%)	13 (6)	12 (6)	1 (3)	0.58
History of Anxiety, N (%)	13 (6)	12 (6)	1 (3)	0.58
History of Other Psychiatric Disorder, N (%)[a]	3 (1)	3 (1)	0 (0)	0.51
Anti-Depressant Medication Use, N (%)	22 (10)	22 (11)	0 (0)	0.09

Values are mean (SD), median (IQR), or N (%).
Abbreviations: BMI = body mass index, FRS = Framingham Risk Score, LDL = low density lipoprotein.
Bold value signifies $p < 0.05$.
[a] Other psychiatric disorders include post-traumatic stress disorder (N = 2) and obsessive-compulsive disorder (N = 1).

survival in subjects stratified by AmygA and adiposity. For multivariable models, important covariates were selected a priori and included age, sex, race, measures of adiposity, AmygA, baseline FBG, cardiovascular risk factors, history of pre-diabetes, and statin use. Time between imaging and date of event or last follow-up was entered into models where appropriate. Missing data were dropped from relevant sub-analyses; imputation was not employed. All tests were two-sided with statistical significance defined as $p < 0.05$.

3. Results

3.1. Descriptives

Baseline characteristics, including pre-existing psychiatric conditions, are provided in Table 1. Two hundred thirty-two (232) individuals without baseline DM were followed longitudinally and provided adequate follow-up data to assess the development of incident DM (Fig. 1A). Twenty-one (21) (9.1%) and 30 (12.9%) of these patients developed type 2 DM over two and five years, respectively (running total). At baseline, 114 individuals (49.1%) met criteria for pre-diabetes within the two years preceding index imaging. A total of 119 individuals (51.3%), 18 of which did not have pre-diabetes at baseline, had evidence of pre-diabetes within two years of follow-up, and a total of 129 individuals (55.6%), of which 36 did not have pre-diabetes at baseline, had evidence of pre-diabetes within five years of follow-up. Glycemic data were not available for the full five years for 86 subjects.

Table 2
Clinical predictors of incident type 2 diabetes.

Predictor	Covariates	HR	95% CI		p
Age	None	1.02	0.98,	1.05	0.38
Sex	None	2.29	0.95,	5.53	0.07
Caucasian	None	1.16	0.27,	4.97	0.84
Hypertension	None	0.89	0.35,	2.30	0.81
Baseline Glucose	None	1.87	1.59,	2.22	**< 0.001**
Statin	None	1.96	0.76,	5.05	0.16
BMI	None	1.27	0.85,	1.92	0.24
Baseline VAT	None	1.47	0.94,	2.31	0.09
Baseline SAT	None	0.83	0.50,	1.38	0.46
Baseline VAT: SAT	None	1.27	0.83,	1.97	0.27
Total Adipose Tissue	None	1.05	0.66,	1.68	0.84
AmygA	None	1.46	1.08,	1.97	**0.01**
AmygA	Age	1.43	1.04,	1.96	**0.03**
AmygA	Baseline glucose	1.35	1.02,	1.77	**0.03**
AmygA	BMI, age, race, history of pre-diabetes	1.40	1.01,	1.95	**0.04**
AmygA	BMI, age, race, history of pre-diabetes, statin use	1.40	1.01,	2.95	**0.04**
AmygA	BMI	1.46	1.08,	1.98	**0.01**
AmygA	VAT	1.44	1.01,	2.06	**0.046**

Abbreviations: AmygA: amygdalar activity, BMI: body mass index, CI: confidence interval, DM: diabetes mellitus, HR: hazard ratio, SAT: subcutaneous adipose tissue, VAT: visceral adipose tissue.
Baseline amygdalar activity tested as a continuous variable.
Bold value signifies $p < 0.05$.

3.2. Amygdalar activity independently predicts incident diabetes after multivariable adjustments

Baseline AmygA, as a continuous variable, associated with incident type 2 DM risk in univariable analysis (HR [95% CI]: 1.46 [1.08, 1.97], p = 0.01, Table 2, Supplemental Table A1). This remained significant after adjusting for baseline blood glucose concentrations (1.35 [1.02, 1.77], p = 0.03), age (1.43 [1.04, 1.96], p = 0.03), or other well-recognized predictors of DM (viz., history of pre-diabetes, sex and baseline VAT). Additionally, AmygA predicted DM in the subset of 118 individuals without a history of impaired glucose tolerance (2.66 [1.23, 5.79], p = 0.01) and in the subset of 178 individuals without exposure to systemic corticosteroids within two years prior to imaging (1.43 [1.03, 1.99], p = 0.03).

In a post-hoc analysis, we additionally observed an association between baseline AmygA and the timing of incident type 2 DM. Individuals who developed DM sooner had higher AmygA than those who later- or never developed DM (p = 0.02, Supplemental Fig. A1). Additionally, AmygA predicted type 2 DM, whether events were assessed at one, two, three, or five years after index imaging (Supplemental Table A2).

The association between AmygA and type 2 DM became especially robust when AmygA was dichotomized as: < vs. ≥ sample median. Individuals with higher AmygA had an approximately 5-fold increased risk for incident DM (4.91 [1.65, 14.60], p = 0.004, Figs. 2 and 3A,

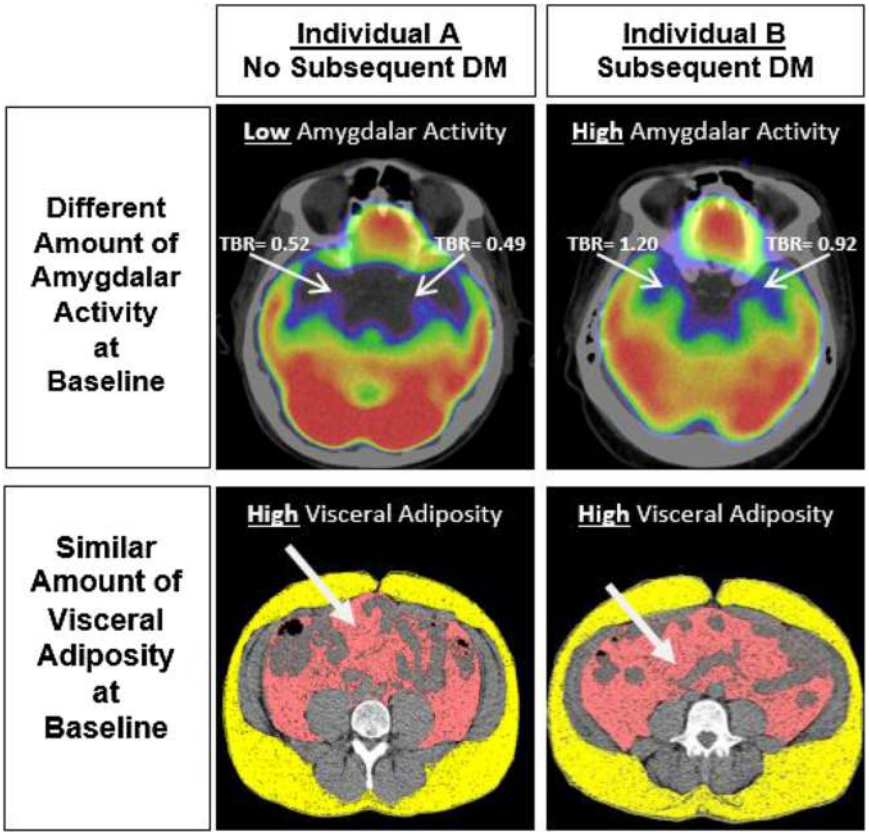

Fig. 2. Imaging of amygdalar activity and adiposity in individuals with vs. without subsequent type 2 diabetes. Axial views of amygdala (upper right and left ^{18}F-FDG-PET/CT images) and adipose tissues are shown (lower right and left CT images) from two individuals. Both individuals were obese and had similar amounts of visceral adipose tissue. However, AmygA was increased in the individual who developed DM (right) compared to the individual who did not (left).
Abbreviations: DM: diabetes mellitus, TBR: target-to-background ratio.

Table 3, Supplemental Fig. A2). That risk remained significant in multivariable models that adjusted for traditional risk factors for DM, baseline glycemic variables, or measures of adiposity (Table 3).

Notably, high AmygA significantly increased risk for DM even among the sub-groups of individuals at increased risk for type 2 DM due to greater adiposity (Table 3) as assessed as: A) baseline BMI ≥ 25 kg/m^2 (6.37 [1.44, 28.23], p = 0.02) or B) ≥ median baseline VAT (6.57 [1.42, 30.43], p = 0.02). Those associations remained significant in multivariable analyses. Additionally, high AmygA predicted incident DM in individuals who were not obese (BMI < 30 kg/m^2) at baseline (6.59 [1.45, 30.10], p = 0.02). This finding remained significant after adjusting for baseline VAT (6.61 [1.44, 30.36], p = 0.02, Supplemental Fig. A3).

3.3. Synergy between amygdalar activity and adiposity in determining diabetes risk

We next explored the potential synergy between AmygA and adiposity on type 2 DM risk. For these analyses, AmygA was again dichotomized by the median value. Adiposity measures were dichotomized similarly. This two-by-two factorial analysis placed individuals into one of four groups: A) low adiposity, low AmygA, B) low adiposity, high AmygA, C) high adiposity, low AmygA, and D) high adiposity, high AmygA. The analysis was repeated three times, each using a different measure of adiposity: A) BMI (< 25 or ≥ 25 kg/m^2), B) baseline VAT (< or ≥ sample median), and C) change in VAT (< or ≥ sample median). Across each of these analyses (Fig. 3B–D), low AmygA associated with a low risk of DM, regardless of adiposity. Conversely, the greatest DM risk was observed in individuals with both high AmygA and high adiposity, suggesting that high AmygA and high adiposity may act synergistically to increase type 2 DM risk.

3.4. Validation in a separate cohort

We tested the relationship between AmygA and metabolic parameters in a separate biomarker study of 87 prospectively recruited individuals (characteristics in Supplemental Table A2). Subjects were designated as having "higher" vs. "lower" AmygA (highest tertile vs. pooled middle and lower tertiles). Higher AmygA associated with higher levels of several inflammatory biomarkers: CCL5 (p = 0.04), CXCL5 (p = 0.03), and ENRAGE (neutrophil-derived extracellular newly identified receptor for advanced glycosylation end products) (p = 0.02). Furthermore, higher AmygA associated with greater insulin resistance, measured as homeostatic model assessment of insulin resistance (HOMA-IR) (p = 0.02). Higher AmygA also associated with lower levels of the beneficial adipokine, adiponectin (p = 0.005). These findings are shown in Supplemental Fig. A4 and Table A4.

4. Discussion

This study employed simultaneous imaging of brain and adipose tissues and leveraged longitudinal clinical assessments to demonstrate that increased neurobiological activity related to stress (i.e., AmygA) confers a substantially increased risk of developing type 2 DM in the future. The association between higher AmygA and DM risk remained robust after accounting for type 2 DM risk factors, including glucose measures, demographic data, and imaging measures of adiposity. Lower AmygA associated with a low risk of DM regardless of adiposity. Conversely, individuals with both high AmygA and high adiposity had a markedly increased risk of type 2 DM, suggesting that increased AmygA may combine with adiposity via a "two-hit" mechanism to promote DM. Accordingly, these findings identify the amygdala, a neural region involved in the neurobiology of stress, as a previously underappreciated, yet potentially important participant in the mechanisms that provoke type 2 DM in humans.

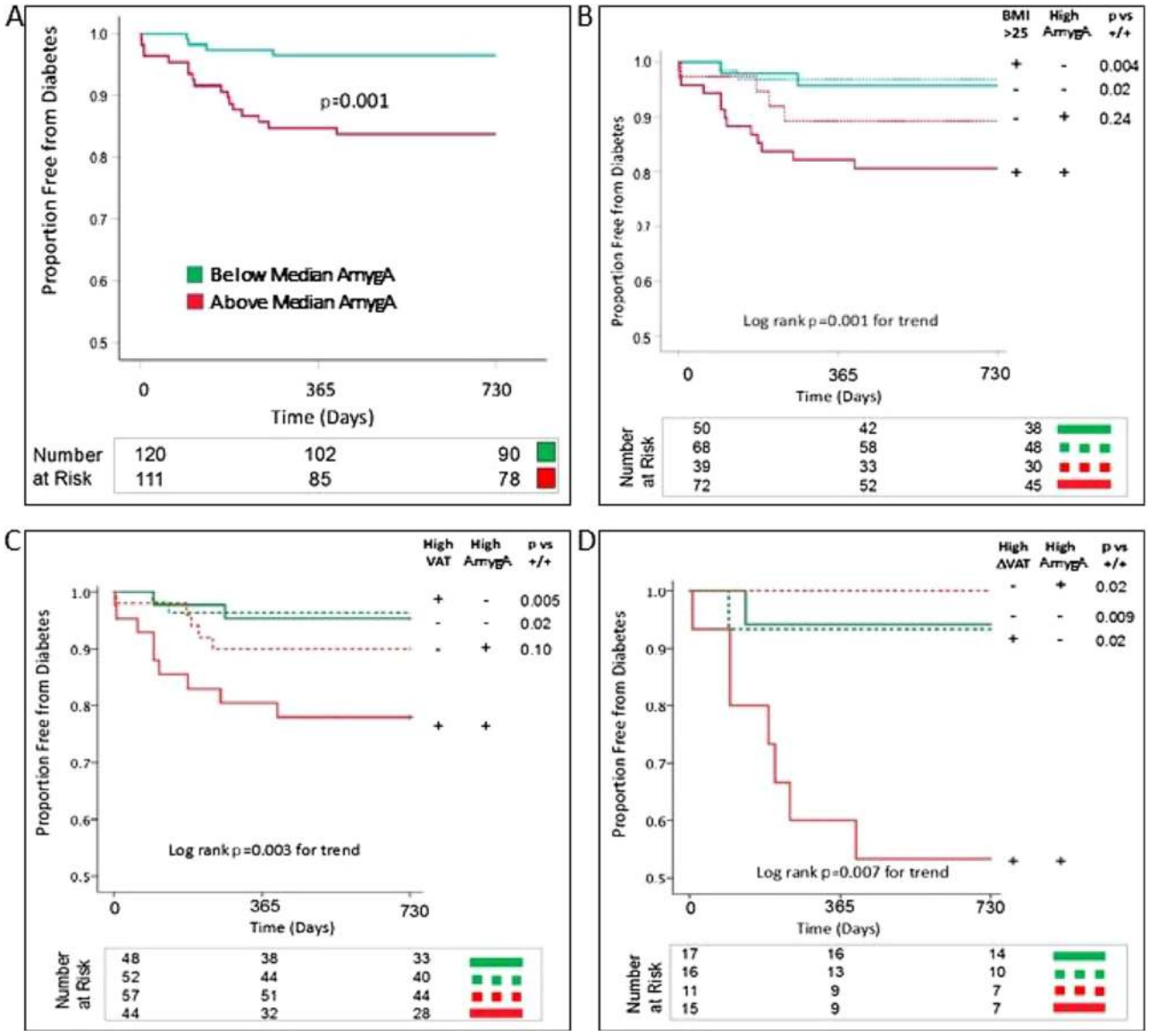

Fig. 3. Kaplan-Meier curves showing type 2 diabetes-free survival as a function of amygdalar activity alone (A) and as a function of both amygdalar activity and (B) body mass index, (C) visceral adipose tissue, and (D) subsequent change in visceral adipose tissue.

A. Subjects were categorized by baseline AmygA [< (green) or ≥ (red) sample median]. Those with high AmygA had substantially higher incidence of DM. The total number of subjects assessed is 231, as one individual did not provide cerebral background data to calculate the pre-specified AmygA endpoint.

B. Subjects were categorized by baseline AmygA [< (green) or ≥ (red) sample median] and baseline BMI [< (dashed) or ≥ (solid) 25 kg/m^2]. Those with high AmygA and BMI had substantially higher incidence of DM. The total number of subjects assessed is 229, as one individual did not provide cerebral background data to calculate the pre-specified AmygA endpoint, and two subjects did not provide height data to calculate BMI.

C. Subjects were categorized by baseline AmygA [< (green) or ≥ (red) sample median] and baseline VAT [< (dashed) or ≥ (solid) sample median]. Those with high AmygA and VAT had substantially higher incidence of DM. The total number of subjects assessed is 201, as one individual did not provide cerebral background data to calculate the pre-specified AmygA endpoint, and 30 individuals did not provide data for measurement of VAT.

D. Subjects were categorized by baseline AmygA [< (green) or ≥ (red) sample median] and subsequent change in VAT [< (dashed) or ≥ (solid) sample median]. Those with high AmygA and change in VAT had substantially higher incidence of DM. Serial VAT measurements were available in 59 individuals.

Abbreviations: AmygA: amygdalar activity, BMI: body mass index, DM: diabetes mellitus, VAT: visceral adipose tissue.

4.1. Mechanistic insights

It has been hypothesized for millennia that psychological stress associates with physical maladies. Recently, stress has gained attention as a risk factor for metabolic disease (Kumari et al., 2004; Mooy et al., 2000). Clinical guidelines currently acknowledge that psychosocial stress may complicate DM care (Chida and Hamer, 2008). Our results provide novel evidence implicating the role of increased AmygA in predicting incident type 2 DM. Although it has been reported that individuals with overt DM are prone to have structural abnormalities of the amygdala (den Heijer et al., 2003), the role of AmygA in potentiating future DM risk was previously unknown. The current study, for the first time in animals or humans, identifies the amygdala as a brain region that may link stressors to the subsequent development of type 2 DM. From these observations, we hypothesize that the amygdala may be a pathobiological contributor to metabolic disorders. Stimulation of the amygdala activates both the hypothalamic-pituitary-adrenal axis (HPAA) and the sympathetic nervous system (the latter through the amygdala's projections to the brainstem) (Drevets et al., 2002; LeDoux et al., 1988). Importantly, sympathetic efferents to the bone marrow stimulate turnover and release of immune cells, which contribute to systemic inflammation and modify the metabolic milieu.

Table 3
Amygdalar activity vs. incident type 2 diabetes after multivariable adjustments.

Predictor	Covariates	Full Cohort		Subgroup Analysis: Individuals with BMI ≥ 25 kg/m^2 at baseline		Subgroup Analysis: Individuals with ≥ median VAT at baseline	
		HR (95% CI)	p-value	HR (95% CI)	p-value	HR (95% CI)	p-value
Above vs. Below Median Amygdalar Activity	None	4.91 (1.65, 14.60)	**0.004**	6.37 (1.44, 28.24)	**0.02**	6.57 (1.42, 30.43)	**0.02**
	Age	4.78 (1.60, 14.26)	**0.005**	6.20 (1.39, 27.54)	**0.02**	6.18 (1.35, 28.24)	**0.02**
	Sex	5.69 (1.90, 17.01)	**0.002**	8.35 (1.88, 37.12)	**0.005**	6.90 (1.49, 31.90)	**0.01**
	Baseline Fasting Blood Glucose	3.82 (1.64, 8.91)	**0.002**	6.81 (1.52, 30.58)	**0.01**	4.98 (1.08, 23.08)	**0.04**
	Maximum Blood Glucose 2 years prior to imaging	4.52 (1.51, 13.54)	**0.007**	6.04 (1.35, 26.98)	**0.02**	6.87 (1.47, 32.20)	**0.01**
	Average Blood Glucose over 2 years prior to imaging	4.01 (1.33, 12.10)	**0.01**	4.86 (1.08, 21.95)	**0.04**	4.90 (1.02, 23.59)	**0.048**
	History of Pre-Diabetes	4.01 (1.33, 12.07)	**0.01**	5.36 (1.18, 24.34)	**0.03**	5.13 (1.05, 24.93)	**0.04**
	DM Risk Factors[a]	4.95 (1.67, 14.79)	**0.004**	6.68 (1.51, 29.62)	**0.01**	6.38 (1.38, 29.53)	**0.02**
	Cardiac Risk Factors[b]	5.91 (1.97, 17.70)	**0.002**	9.91 (1.47, 30.58)	**0.004**	6.70 (1.47, 30.58)	**0.01**
	Statin Use	5.01 (1.69, 14.90)	**0.004**	6.52 (1.47, 28.91)	**0.004**	7.41 (1.59, 14.90)	**0.01**
	BMI	4.87 (1.64, 14.52)	**0.004**	6.67 (1.50, 29.64)	**0.01**	6.45 (1.36, 34.56)	**0.02**
	VAT	4.10 (1.35, 12.46)	**0.01**	5.37 (1.18, 24.59)	**0.03**	5.98 (1.29, 27.79)	**0.02**
	SAT	4.00 (1.32, 12.15)	**0.01**	5.11 (1.12, 23.24)	**0.04**	7.41 (1.57, 34.96)	**0.01**
	VAT:SAT	3.97 (1.31, 12.09)	**0.02**	5.05 (1.10, 23.08)	**0.04**	6.78 (1.46, 31.40)	**0.01**
	Total Adiposity	4.03 (1.33, 12.25)	**0.01**	5.30 (1.16, 24.19)	**0.03**	6.68 (1.42, 31.49)	**0.02**
	History of Prior Cancer	4.84 (1.63, 14.39)	**0.005**	6.43 (1.45, 28.51)	**0.01**	6.47 (1.39, 30.08)	**0.02**
	History of Depression/Anxiety	4.44 (1.48, 13.27)	**0.008**	5.68 (1.27, 25.37)	**0.02**	5.76 (1.22, 27.12)	**0.04**
	History of Corticosteroid Use	4.91 (1.65, 14.61)	**0.004**	6.79 (1.64, 32.33)	**0.009**	6.53 (1.41, 30.25)	**0.02**

231 individuals were included in the outcomes cohort analysis, as one individual did not provide background cerebral data to calculate the pre-specified amygdalar activity endpoint.
Abbreviations: BMI: body mass index, CI: confidence interval, HR: hazard ratio, SAT: subcutaneous adipose tissue, VAT: visceral adipose tissue.
Bold value signifies $p < 0.05$.
[a] Age, race, history of pre-diabetes, and BMI entered as cofactors.
[b] Age, smoking, hypertension, dyslipidemia, statin use and family history entered as cofactors.

In animal studies, chronic stress promotes systemic inflammation (Heidt et al., 2014) and increases VAT (Bartolomucci et al., 2009), which both lead to greater insulin resistance (Karagiannides et al., 2014; Uchida et al., 2012). We observed associations between AmygA and markers of inflammation and metabolic impairment. Together these factors could combine to promote the development of type 2 DM (Supplemental Fig. A5).

4.2. Observed incident diabetes not solely the result of subsequent gains in adiposity

This study's finding that increased AmygA confers DM risk independent of subsequent increases in VAT is particularly notable. This result suggests that the association between AmygA and type 2 DM is unlikely to be mediated solely by behavioral changes (e.g., increased caloric intake and decreased physical activity), which collectively

increase adiposity. If altered health behaviors after baseline imaging were indeed the primary reason for the association between AmygA and type 2 DM, then adjusting for an important consequence (i.e., subsequent increases in adiposity) should have attenuated the relationship between AmygA and DM. However, AmygA remained a strong predictor of DM, even after such adjustments, suggesting that these findings extended beyond the effects of altered health behaviors alone. Moreover, in individuals with increases in adiposity after baseline imaging, AmygA remained a particularly potent predictor of type 2 DM risk.

4.3. Limitations

Our study has limitations. Questionnaire-based assessments of stress were not employed in this study, which limits the ability to directly link stress to AmygA in this population ; however, we have previously shown that AmygA closely correlated with a measure of perceived stress (Tawakol et al., 2017). Outcomes study subjects were identified from a database of patients who had undergone clinical ^{18}F-FDG-PET/CT screening for cancer, possibly limiting the generalizability of the findings. Because the outcomes study utilized imaging and laboratory data obtained during clinical care, it is subject to the inherent limitations of such a design. However, the biomarker study, which included clinically stable patients without suspected malignancy, yielded confirmatory findings that provide confidence in the outcomes study's results by demonstrating a similar cross-sectional relationship between increased AmygA and metabolic impairment. Additionally, due to the incomplete coverage of the brain and the limited resolution of clinical whole-body ^{18}F-FDG-PET/CT imaging, we were unable to assess other brain regions involved in stress perception (e.g., the hippocampus and insular cortex). Finally, although it is hypothesized that this neural-metabolic mechanism acts through effects on the HPAA and sympathetic nervous system, direct measurements of these pathways were not performed. Similarly, an evaluation of health behaviors during follow-up was not performed in this retrospective study. Nonetheless, this study's limitations are counterbalanced by important innovations, including the use of unique hybrid imaging for the simultaneous quantification of AmygA and VAT, to identify a compelling link between neurobiological activity and DM.

4.4. Future perspectives

Future studies should test whether modifying the observed central neural-metabolic mechanism may decrease the burden of metabolic disease. Studies are needed to clarify the hypothesized roles of the HPAA and sympathetic nervous system. Although pharmacotherapies targeting these pathways should be pursued, non-pharmacologic approaches may also be fruitful. For example, stress management and mindfulness-based stress reduction interventions have demonstrated positive effects on DM (Medina et al., 2017; Surwit et al., 2002). Our results suggest value in screening for metabolic impairment in individuals with high stress who are at elevated risk of developing type 2 DM, and who may benefit from stress reduction approaches.

5. Conclusions

This study demonstrates, for the first time, a relationship between amygdalar metabolic activity and subsequent risk of type 2 DM. We observed that high amygdalar activity strongly and independently increases the risk of DM, whereas lower amygdalar activity associates with lower risk of DM, even among overweight and obese individuals. These findings support a "two-hit" model in which adiposity and a neurobiological response to stress combine to precipitate DM, and, furthermore, highlight a path against which novel therapeutic strategies may be applied. Moreover, these results further underscore the need to consider psychosocial stress when assessing individuals at risk for type 2 DM.

Declaration of interests

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MTO, AI, JTG, SKG, RKP, and AT contributed to study design. BH and AT performed statistical analysis. AI, BT, and YW provided image analysis and clinical adjudication. AB contributed biomarker data. Expertise was provided by ZAF and AT for PET/CT imaging, SKG and JL for metabolic diseases, KCK and RKP for stress conditions, and LS for neuroimaging. MTO and AT led manuscript preparation and revisions. AT performed leadership duties and is the guarantor of this work. He had full access to the study data and takes responsibility for its integrity and the accuracy of the analysis. All authors participated in preparation and revisions and approved this manuscript for submission. MTO and AI contributed equally and are co-first authors.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.psyneuen.2018.09.024.

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