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Central adiposity and brain vulnerability in mid-life: Evidence for early risk

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Central adiposity and brain vulnerability in mid-life: Evidence for early risk

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Dedication

This is dedicated to my husband, Richard Peter Trevivian. Thank you for all your love and support.

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Central adiposity and brain vulnerability in mid-life: Evidence for early risk

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This set of projects focused on visceral fat, measured using proximal and direct methods. Specifically, I was interested in the effects of visceral fat on brain structure and integrity in middle age. Study 1 looked at waist to hip ratio (WHR) as a proximal measure of visceral fat and used statistical mediation to directly examine a possible mechanism behind the relationship between visceral fat and cognitive decline. Reductions in executive function seen in middle-aged adults with high visceral fat were found to occur in the context of lowered serum brain derived neurotrophic factor (BDNF) a key neurotrophin involved in synaptic plasticity as well as neuronal regeneration. Study 2 utilized Dual Energy X Ray Absorptiometry (DXA) to directly estimate visceral fat mass and volume as well as thickness of the cortical mantle in middle age. High-resolution Magnetization Prepared Rapid Acquisition Gradient Echo (MPRAGE) images were used. High visceral fat was found to predict increased thickness in the posterior cingulate cortex independently of age and cardiovascular risk in a cognitively intact middle-aged sample. Study 3 examined changes in concentrations of crucial cerebral metabolites in the posterior cingulate cortex among individuals with high visceral fat. Results indicated that visceral fat predicted reduced concentrations of N Acetyl Asparate, a marker of neuronal viability

and increased concentrations of myo-inositol, a glial marker that is implicated in a number of disease states including prodromal Alzheimer's Disease and Multiple Sclerosis. Collectively, the 3 studies highlight important evidence for early brain vulnerability even in cognitively intact middle- aged adults with high levels of cognitive reserve. An important next step would be to examine modifiable mediators of these relationships, such as inflammation and BDNF so that targeted interventions may be developed.

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Central adiposity and brain vulnerability in midlife: Evidence for early risk

The number of individuals who have been classified as overweight or obese has doubled in the past two decades, currently encompassing almost two thirds of the adult population in the United States (NCHS, 2006). Obesity has been implicated in several negative outcomes, including increased risk for stroke, gall bladder disease, cancer and overall mortality (Kopelman *et al.*, 2000). Furthermore, recent evidence has shown a similar deleterious effect of obesity on the brain (Gustafson *et al.*, 2004). Obesity at midlife leads to increased risk for dementia (Gustafson *et al.*, 2003) and poorer performance in cognitive tasks, particularly those that measure executive function (Stingl, 2012). Furthermore, obesity has been associated with reduced working memory related functional activation in the brain (Gonzales *et al.*, 2014), perturbed concentrations of crucial cerebral metabolites (Gonzales *et al.*, 2012), increased white matter volume (Haltia, 2007), lower grey matter density (Pannacciulli *et al.*, 2006) and lower brain volumes in patients with Alzheimer's Disease and mild cognitive impairment (Ho *et al.*, 2010). Given the rising prevalence of obesity, coupled with the fact that neurodegeneration is irreversible, identification of the underlying mechanisms that drive the detrimental effect of obesity on the brain is a public health imperative.

Published research has shown that the distribution of adiposity may be a more important predictor of cognitive vulnerability than body mass (Cereda *et al.*, 2007). Adipose tissue that is distributed along the arms and legs of the body is composed of subcutaneous fat, which accumulates under the skin and represents approximately 80% of total body fat (Ibrahim, 2010). In contrast, adipose tissue that is distributed along the

waistline is indicative of visceral fat (Ibrahim, 2010), which is metabolically active and a more significant predictor of cognitive decline and dementia compared to body mass index (BMI) (Cereda *et al.*, 2007; Kanaya *et al.*, 2009; Kerwin *et al.*, 2011; Whitmer *et al.*, 2007; Whitmer *et al.*, 2008). While some studies have examined the direct effect of central adiposity on cognition in older adults (Whitmer *et al.*, 2008), there have been relatively few such studies with younger populations without concomitant age related cognitive decline and neurological disease comorbidity. The aim of this dissertation is to explore the relationship between visceral fat and brain vulnerability in middle age. The basic thesis is that visceral fat is associated with significant cognitive decline, changes in structural integrity of the brain, as well as reduced neuronal viability and that these changes are seen in cognitively intact, neurologically healthy middle-aged adults. This project includes three studies, each employing a unique methodology and investigating outstanding questions regarding the effect of visceral fat on the brain. Identification of these changes early in life is crucial, as clinically significant cognitive decline is often preceded by a period of latent degenerative changes that manifest in middle aged and younger adults (Gustafson *et al.*, 2008). This body of research is intended as a first step in identifying possible mechanistic pathways through which central adiposity impinges upon central nervous system functioning. In this dissertation, a brief overview of common themes and mechanisms that lead to the hypotheses for the three studies will be provided. More detailed overviews and methodological descriptions specific to each study will follow.

Prevalence and health impact of obesity

In 2004, 71.1 percent of men and 61.4 percent of women aged between 20 and 74 years in the United States met criteria for being overweight or obese according to globally established criteria (BMI ≥ 30) (Ogden *et al.*, 2007). Worldwide, the prevalence for overweight and obesity among adults rose by 27.5% between 1980 and 2013 (Ng *et al.*, 2014). As obesity is associated with a host of adverse health outcomes including increased mortality, risk for cancer, diabetes and gall bladder disease (Kopelman *et al.*, 2000). The total estimated direct and indirect costs of obesity related health problems have been estimated at $117 billion (Wolf *et al.*, 1998). Despite concerted governmental efforts to combat obesity in the United States (Khan *et al.*, 2009), prevalence remains high (Ogden *et al.*, 2014).

Obesity and cognitive function

In addition to documented detrimental effects of obesity on physical health, recent evidence has shown obesity exerts similarly deleterious effects on brain health (Gustafson *et al.*, 2004). Obesity at midlife results in increased risk for dementia 18 -24 years later (Fitzpatrick *et al.*, 2009; Gustafson *et al.*, 2004; Gustafson D, 2003; Kivipelto *et al.*, 2005; Whitmer *et al.,* 2005) and poorer performance in cognitive tasks, particularly those that measure executive function (Stingl *et al.*, 2012). Data from the Baltimore Longitudinal Study of Aging revealed declines in performance on global cognition, memory and executive function tasks in non demented individuals with baseline obesity (Gunstad, 2010). Furthermore, obesity is associated with poorer performance in memory and executive function tasks 4-6 years later (Elias *et al.*, 2003). As cognition is the most useful predictor of late life functional independence and quality of life (Banaszak-Hall *et*

al., 2004; Gaugler *et al.*, 2009), it is imperative to identify the mechanisms through which these obesity related changes occur.

Obesity and brain integrity

It is possible to hypothesize that obesity driven changes in cognitive function are the result of changes to structural integrity of the brain. Obesity has been associated with alterations across multiple indices of brain health including working memory related functional activation in the brain (Gonzales *et al.*, 2014), perturbed concentrations of crucial cerebral metabolites (Gonzales *et al.*, 2012), increased white matter volume (Haltia, 2007), lower grey matter density (Pannacciulli *et al.*, 2006) and lower brain volumes in patients with Alzheimer's Disease and mild cognitive impairment (Ho *et al.*, 2010). Higher BMI is also linked with neuronal and myelin abnormalities in the frontal lobes of cognitively intact elderly participants (Gazdzinski *et al.*, 2010). Furthermore, BMI is negatively correlated with white matter integrity in the fornix and corpus callosum (Stanek *et al.*, 2011; Xu *et al.*, 2013) among otherwise healthy younger adults. However, despite these well established and well regarded findings, some researchers have reported no association between midlife obesity and structural indices of brain health in old age (Albanese *et al.*, 2015). The relationship between being overweight/obese in midlife and brain health is thus complex and warrants further investigation.

Obesity and adipose tissue distribution

Adipocytes, the primary component of adipose tissue, may directly impact cerebral integrity. Adipocytes are metabolically active and are able to modulate

inflammatory and growth factor pathways that affect central nervous system functioning (Gustafson *et al.*, 2010). Adipose tissue increases production of pro-inflammatory cytokines (Fried *et al.*, 1998) that are known to affect brain functioning (Eagan *et al.*, 2012). However, the distribution of adipose tissue appears to be a pertinent predictor of subsequent metabolic and cardiovascular disease than is total adipose mass (Janssen *et al.*, 2004). Adipose tissue distributed around the arms and legs of the body contains subcutaneous fat, which comprises approximately 80% of total body fat (Ibrahim *et al.*, 2010). In contrast, adipose tissue that accumulates around the waistline is indicative of visceral fat, which is metabolically active and may be more detrimental to physical and neuronal health (Ibrahim *et al.*, 2010).

Central adiposity and brain function

It has been suggested that central adiposity is a stronger predictor of subsequent neurocognitive decline than body mass index, or measures of subcutaneous fat (Whitmer *et al.*, 2008). High central adiposity is associated with reduced amounts of circulating leptin (Al Hazzouri *et al.*, 2012), a hormone that is critical in preventing lipotoxicity (Shimabukura *et al.*, 1998). Lipotoxicity is a phenomenon that occurs when lipid intermediates accumulate in non-adipose tissue, resulting in cellular dysfunction and death (Garbarino *et al.*, 2009; Schaffer *et al.*, 2003). In addition, central adiposity and thus visceral fat is associated with established peripheral risk factors for brain vulnerability such as insulin resistance (McKeigue *et al.*, 1991; Raji *et al.*, 2001), inflammation (Fontana *et al.*, 2007) and oxidative damage (Pou *et al.*, 2007).

Central adiposity and insulin resistance

Visceral fat has been correlated with insulin resistance (Raji *et al.*, 2001), a phenomenon that occurs as a result of abnormally inefficient secretion of insulin in response to elevations in blood glucose (de Luca *et al.*, 2008). Insulin resistance is ameliorated by leptin (Cortes *et al.*, 2014), a hormone that is secreted in reduced amounts among individuals with high visceral fat (Cortes *et al.*, 2014). Insulin resistance is known to impinge upon cerebral glucose metabolism (Doyle *et al.*, 1995) and mediate the relationship between BMI and working memory related brain activation (Gonzales *et al.*, 2010). Furthermore, insulin resistance has been demonstrated in Alzheimer's Disease patients (Talbot *et al.*, 2012) and been correlated with amyloidosis in mouse models of Alzheimer's Disease (Ho *et al.*, 2004). Additionally, intranasal insulin administration has

led to improved memory, attention and global cognition in Alzheimer's Disease patients (Craft *et al.*, 2012; Reger *et al.*, 2008). Thus, there appears to be a link between cognition and insulin regulation mechanisms. Taken together, the literature supports a strong correlation between central adiposity driven insulin resistance and later cognitive decline.

Central adiposity and inflammation

Central adiposity is also highly correlated with systemic inflammation in obese adults (de Luca *et al.*, 2008; Fontana *et al.*, 2007). Higher levels of pro-inflammatory cytokines such as interleukin 1 (IL-1) cause activation of inducible nitric oxide synthase astrocytes, which could indirectly potentiate N-Methyl-D-aspartate (NMDA) induced neurotoxicity (Hewett *et al.*, 1994), ultimately manifesting in neuronal death. In fact, it has been hypothesized that late life cognitive decline in individuals with the metabolic syndrome occurs primarily in the context of systemic inflammation (Yaffe *et al.*, 2004). Furthermore, inflammation has also been associated with changes in concentrations of crucial cerebral metabolites (Eagan *et al.*, 2012) and cortical thinning in middle aged adults (Kaur *et al.*, 2014). It is thus reasonable to suggest that visceral fat could lead to changes in neuronal structure and viability and eventually cognitive decline by fostering neuroinflammation.

Central adiposity and Brain Derived Neurotrophic Factor (BDNF)

BDNF is a key neurotrophin responsible for neuronal regeneration and survival, as well as synaptic plasticity (Mattson, *et al.,* 1997). Mouse models have depicted higher rates of obesity and hyperactivity as a result of conditionally deleted BDNF in the brain (Kernie *et al.*, 2000; Kerwin *et al.*, 2011). Presence of the met allele in Val66Met, a

genetic polymorphism associated with lower levels of BDNF, is also significantly associated with poorer performance on cognitive tests, particularly those that measure executive function in obese individuals (Marques-Iturra *et al.*, 2014). High BDNF is also correlated with low insulin resistance (Levinger *et al.*, 2008), a demonstrated consequence of high central adiposity (Raji *et al.*, 2001). Infusion of BDNF into the lateral ventricles of diabetic mice also normalizes glucose regulation (Nakagawa *et al.*, 2002). The BDNF pathway is thus highly linked to cerebral insulin regulation mechanisms and it is possible that high central adiposity may disrupt BDNF proliferation in the central nervous system, resulting in structural brain changes and reduced cognitive function.

Measuring central adiposity

Indirect methods of measuring central adiposity include waist circumference (Gonzales *et al.*, 2014) and the ratio of waist to hip circumference (WHR) (Brook *et al.*, 2001). Both of these measures have been shown to be more predictive of cognitive dysfunction and dementia than BMI (Cereda *et al.*, 2007; Kanaya *et al.*, 2009) or indices of cardiovascular disease (Brook *et al.*, 2001; Terry *et al.*, 1992). As these methods are noninvasive and inexpensive, they are widely used as an estimate of central adiposity in clinical research.

Researchers have also started to directly examine central adiposity through computed tomography (CT) (Yoshizumi *et al.*, 1999), magnetic resonance imaging (MRI) (Janssen *et al.*, 2002) and dual energy X-ray absorptiometry (DXA) (Kaul *et al.*, 2012). While indirect methods of measuring central adiposity have shown some

prognostic value, research has shown that more direct methods of measuring visceral fat have better utility in predicting neurodegeneration in elderly populations (Isaac *et al.*, 2011). In particular, DXA has proved a useful tool for accurately measuring visceral fat mass and volume (Clasey *et al.*, 1999). As DXA is quick, relatively inexpensive and noninvasive with results comparable to that of CT (Kaul *et al.*, 2012; Xia *et al.*, 2014), it is a highly desirable method for the direct measurement of central adiposity.

Measuring brain vulnerability

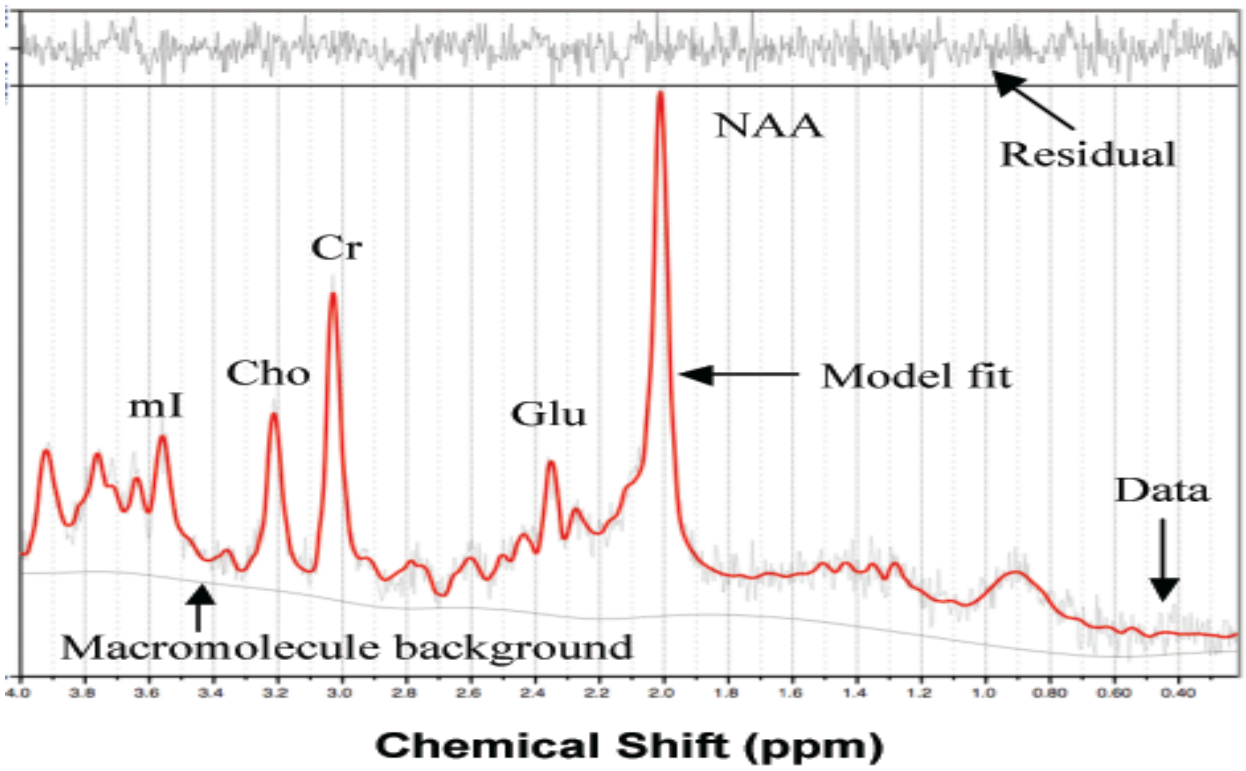
Brain vulnerability has been assessed in the literature in a number of ways. Performance on cognitive tests is a direct way of examining brain behavior relationships (Lezak *et al.*, 2004). Cognitive function is also the most salient predictor of quality of life and independence in late life (Banaszak-Hall *et al.*, 2004; Gaugler *et al.*, 2009). However, clinically significant cognitive decline is often preceded by a latent period of neurodegenerative changes in younger adults and middle age (Gustafson *et al.*, 2008). As such, it is pertinent to examine other methods with higher sensitivity to detect early alterations in structural and functional capacity of the brain, so targeted interventions may be developed.

Structural magnetic resonance imaging has been utilized as an intuitive tool to directly examine brain vulnerability. Structural changes in brain volume and volume of subcortical key structures are hallmarks of neurodegenerative conditions such as Alzheimer's Disease (Fox *et al.*, 1997). Previous work has highlighted changes in thickness of the cerebral cortex as a result of metabolic disorders including obesity

(Leritz *et al.*, 2011) and in asymptomatic amyloid-positive adults (Dickerson *et al.*, 2009).

Rapidly developing improvements in magnetic resonance spectroscopy (MRS) imaging techniques has afforded researchers with the opportunity to examine neurochemicals and their metabolites *in vivo* (Ross *et al.*, 2004). N-Acetyl Aspertate (NAA) for example, is a neurometabolite found only in the adult central nervous system (Simmons *et al.*, 1991; Urenjak *et al.*, 1992). It is thus used as a marker of neuronal density, although it would be more accurately described as a marker of neuronal viability as changes in NAA are not always irreversible (De Stefano *et al.*, 1995). NAA is reduced in disease states where neuronal loss is evident such as tumors (Falini *et al.*, 1996) and in sedentary middle aged adults (Gonzales *et al.*, 2013). NAA can be replenished in disease states such as multiple sclerosis (Mostert *et al.*, 2006) and bipolar disorder (Moore *et al.*, 2000) through therapeutic infusion of drugs such as lithium and fluoxetine. Figure 1 depicts the concentrations of NAA as well as other cerebral metabolites of neurobiological significance in the adult central nervous system. These concentrations are represented by the peaks, with residual variance being removed.

Figure 1: Example ^{1}H MR Spectrum of adult brain.



Overview of the proposed studies

This project includes three studies examining the effects of central adiposity as a proxy for visceral fat on brain structure and function, each employing a unique methodology. Central adiposity is measured using WHR as a proxy (Study 1) and DXA as a more direct measure (Studies 2 and 3). Brain function was assessed in study 1 using performance on cognitive assessment. Studies 2 and 3 examined the direct effect of visceral fat on brain structure, operationalized as cortical thickness measured through MRI and neuronal viability assessed through the concentrations of cerebral NAA using

MRS. All studies included neurologically healthy, middle aged participants (40-60 years) with no current cognitive impairment.

Data were drawn from two different projects: the Physical Fitness, Cardiovascular and Brain Health Study (FIT, Study 1) and the Neural Consequences of Metabolic Syndrome (MetS, Studies 2 and 3). Major strengths of both datasets include an ethnically representative sample comparable to that of the state of Texas (44.4 % Non-Hispanic Caucasian; 38.2% Hispanic) and comprehensive assessments of health, including body composition using DXA, cognitive function and neuroimaging.

As the goal of this body of work is to detect and characterize early markers of brain vulnerability, outcome variables for each study were chosen in sequence of sensitivity to early adiposity driven changes. Study 1 examines adiposity related changes in cognitive performance, focusing on executive function measures. Previous research has shown that obese individuals are more likely to perform poorly on tests designed to measure executive function (Elias *et al.*, 2003). Furthermore, we have previously demonstrated diminished working memory related functional activation in individuals with higher waist circumference (Gonzales *et al.*, 2014). We also attempt to elucidate possible mechanisms driving adiposity related changes in executive function, using serum levels of BDNF as a mediator of the relationship between BDNF and executive function. We use both traditional causal steps and nonparametric bootstrapping to answer the following questions 1) Is central adiposity related to poorer performance on measures of executive function in middle age and 2) Does circulating BDNF mediate this relationship?

Study 2 examines the effect of central adiposity on thickness of the cortical mantle.

Central adiposity was measured using DXA to accurately measure visceral fat mass and volume (Clasey *et al.*, 1999; Kaul *et al.*, 2012). We used an exploratory, whole brain approach to examine the effect of central adiposity on thickness at each vertex of the cortical mantle. This study addresses the question: is central adiposity related to structural changes in the cortical mantle in middle age?

Study 3 explores the effect of central adiposity on neuronal viability. Proton magnetic resonance spectroscopy (^{1}H-MRS) will be used to answer the question: is central adiposity related to reductions in cerebral concentrations of NAA? Taken together, the three studies will elucidate different aspects of central nervous system functioning potentially disrupted by abdominal obesity.

STUDY 1: SERUM BRAIN-DERIVED NEUROTROPHIC FACTOR MEDIATES THE RELATIONSHIP BETWEEN ABDOMINAL OBESITY AND EXECUTIVE FUNCTION IN MIDDLE AGE

The number of people being classified as obese or overweight has increased exponentially in the last 20 years in the United States, encompassing approximately two thirds of the adult population (NCHS, 2006). Obesity has been implicated in several adverse health outcomes, including diabetes, cardiovascular and gall bladder diseases, cancer and overall mortality (Kopelman, 2000). Recent evidence has shown similar detrimental effects of obesity on the brain (Gustafson *et al.*, 2004; Whitmer *et al.*, 2008). Being classified as obese is linked to increased risk of dementia (Gustafson *et al.*, 2003; Whitmer *et al.*, 2007) and poorer performance on cognitive tasks (Stingl *et al.*, 2012), particularly tasks that evaluate executive function (Elias *et al.*, 2003). Data from the Framingham heart study highlights deficits in working memory and verbal fluency in obese middle-aged and older men (Elias *et al.*, 2003). However, the mechanisms that lead to this cognitive decline are poorly understood. As neurodegeneration is irreversible, it is crucial to identify the mechanisms underlying this relationship early in life such that targeted preventative measures may be developed and implemented.

The distribution of adipose tissue appears to selectively impact cognitive function (Cereda *et al.*, 2007). In particular, abdominal adiposity is a more salient predictor of cognitive decline and dementia compared with whole-body adiposity as assessed by body mass index (BMI) (Cereda *et al.*, 2007; Kanaya *et al.*, 2009; Kerwin *et al.*, 2011). Higher levels of abdominal fat have been correlated with insulin resistance (Raji *et al.*, 2001), a

phenomenon that has been shown to affect cerebral glucose metabolism (Doyle *et al.*, 1995) and mediate the relationship between BMI and working memory-related functional activation (Gonzales *et al.*, 2010). Insulin resistance is also positively correlated with levels of circulating brain-derived neurotropic factor (BDNF) (Levinger *et al.*, 2008), a key neurotrophin crucial for neuronal regeneration and survival (Mattson *et al.*, 2004). Infusion of BDNF into the lateral ventricles of diabetic mice normalizes glucose regulation (Nakagawa *et al.*, 2002). Mouse models have also depicted higher levels of obesity and hyperactivity as a result of conditional deletion of BDNF in the brain (Kernie *et al.*, 2000; Rios *et al.*, 2001). The BDNF and insulin regulation mechanisms are thus highly linked and central adiposity could cause disruption in BDNF production and distribution in the central nervous system.

BDNF has been correlated consistently to poorer performance in executive function tasks. Serum BDNF levels mediate the positive effects of exercise interventions on task switching in older adults(Leckie *et al.*, 2014). Presence of the Met allele in the val66met polymorphism, a genetic marker linked with lower levels of peripheral and cortical BDNF (Chen *et al.*, 2004; Ozan *et al.*, 2010), is associated with poorer performance on tasks of processing speed (Miyajima *et al.*, 2008) in older adults and higher levels of perseveration responses on the Wisconsin Card Sorting Test (Marques-Iturra *et al.*, 2014) in participants aged between 12-40 years. Thus, it is reasonable to hypothesize that adiposity-related cognitive decline occurs in the context of lower BDNF. However, to our best knowledge, no studies have directly addressed this hypothesis. As BDNF levels can be modified through dietary restriction (Duan *et al*, 2001) and regular

exercise (Wrann *et al.*, 2013), it is vital to tease out the impact of BDNF on central nervous system functioning prior to neurodegeneration. This knowledge could promote the development of targeted interventions involving BDNF infusion and incorporation of non-pharmacological interventions on the BDNF into existing cognitive rehabilitation programs.

In the present study, we used a ratio of waist circumference to hip circumference (WHR) as a proxy for central adiposity. Based on published research from the Framingham heart study (Elias *et al.*, 2003; Wolf *et al.*, 2007) , we hypothesized that individuals with higher abdominal adiposity would demonstrate poorer performance on cognitive tests that evaluate different aspects of executive function. We further hypothesized that these relationships would be accounted for (statistically mediated by) serum levels of BDNF. A successful statistical mediation would imply that central adiposity-related declines in executive functioning occur primarily in the context of lower levels of circulating BDNF.

Method

Participants

Sixty-one adults between the ages of 40-60 years were recruited from the community through electronic and print advertisements. All potential participants underwent a telephone screening and completed a medical history questionnaire to establish eligibility. Exclusionary criteria (Gonzales *et al.*, 2013) included a positive medical history for overt coronary artery disease, neurological disease (e.g., stroke, Parkinson's disease, clinically significant traumatic brain injury), major psychiatric illness (e.g. schizophrenia, bipolar disorder) and substance abuse (i.e., diagnosed abuse and/or previous hospitalization for substance abuse). All participants were nonsmokers. Participants who passed the initial screen were enrolled in the study after providing written consent. The ethnic distribution of the participants was: 82.0% - Caucasian, 6.6% - Hispanic, 4.9% - African-American and 6.5% - Other/Did Not Specify.

Procedures

This study was conducted in accordance with the Helsinki Declaration and with approval from the local Institutional Review Board. All volunteers provided written informed consent before enrollment. Participants underwent three separate study visits, a general health assessment, a neuropsychological assessment and a cardiorespiratory fitness assessment.

General health assessment

Participants abstained from caffeine and fasted for at least 12 hours prior to assessment. Waist circumference was measured at the midpoint between the iliac crest

and lower rib during exhalation as recommended by the World Health Organization (W.H.O., 2008) . Hip circumference was measured at the broadest portion around the buttocks. Brachial systolic and diastolic blood pressure was assessed with a semi-automated device (VP-1000plus, Omron Healthcare, Bannockburn, IL) after 15 minutes of rest. A fasting blood sample was also collected from the antecubital vein by venipuncture. Serum was separated within 2 hours of the collection and aliquots were stored at -80°C until later analysis. Fasting levels of glucose, total cholesterol and triglycerides were measured using the standard enzymatic technique. Serum concentration of BDNF was measured using high sensitivity enzyme linked immunosorbent assay (ELISA) kits (R & D Systems Inc, Minneapolis, MN).

Neuropsychological assessment

Participants completed a battery of standard clinical neuropsychological assessments with established reliability and validity (Lezak *et al.*, 2004), details of which have been described elsewhere (Gonzales *et al.*, 2013). Based on published literature linking obesity (Gonzales *et al.*, 2012) and BDNF (Marques- Itarra *et al.*, 2014) to executive function decline, several tests that tap specific domains of executive function were selected for this analysis: Trail Making Test Part B (TMT) time to completion (Reitan, 1958) – a measure of working memory, Controlled Oral Word Associations test (COWA) (Ruff, 1996) – a measure of verbal fluency and Wechsler Adult Intelligence Scale III Digit Span Subtest (Weschler, 1997). Global cognitive function was screened using the Weschler Test of Adult Reading (WTAR) and the Mini Mental State Examination (MMSE) (Lezak, 2004).

Cardiorespiratory fitness assessment

Participants abstained from caffeine and physical exercise for 24 h and fasted for at least 12 h prior to the visit. Maximal oxygen consumption (VO2 max) was assessed with a graded treadmill exercise test during a modified Bruce protocol. Following a 5-min warm up period, participants ran or walked at a speed that corresponded to 60–70% of their age-predicted maximal heart rate. The treadmill slope was increased 2% every 2 min until volitional exhaustion. Oxygen consumption (indirect calorimetry via respiratory gas measurements; Physio-Dyne, Quogue, NY) and heart rate were measured throughout the protocol. At the end of each stage, participants rated their perceived exertion using the original Borg scale.

Statistical analyses

Statistical analyses were conducted in three steps: first, associations between WHR and performance on each executive function test were examined using linear regressions. Clinically relevant covariates (age, years of education, systolic and diastolic blood pressure, total concentrations of glucose, total cholesterol and cardiovascular fitness) were included in these analyses. To control for multiple comparisons, a Sidak-adjusted two-tailed α-level of 0.01 was used (Sidak, 1967) here. Associations between performance on the one executive function test with significant adiposity effects (COWA) and serum BDNF levels were then assessed using linear regression. Finally, a mediation analysis was performed to test if the association between central adiposity and performance on the COWA is attenuated when considering the role of serum BDNF. For the single mediation analysis, a less conservative α-level of 0.05 was used.

Mediation was assessed using both the traditional causal steps approach and non-parametric bootstrapping procedures (Preacher *et al.*, 2004). The causal steps approach posits that four conditions must be met to determine mediation: 1) a significant relationship between the independent variable (WHR) and the dependent variable (executive function), a significant relationship between the independent variable and the potential mediator (BDNF), 3) a significant relationship between the potential mediator and the dependent variable and 4) a non-significant relationship between the independent variable and the dependent variable after controlling for the potential mediator. An additional assessment on the significance of the mediation model was conducted by using confidence intervals obtained through Preacher and Hayes bootstrapping method for assessing indirect effects (Preacher, 2004). A 95% confidence interval that does not include 0 was used as the criterion for significance. All statistical analyses were carried out using IBM SPSS 22.0 software (SPSS Inc.).

Results

Mean values for demographic and physiological characteristics as well as standard deviations are presented in Table 1.

Table 1: Selected demographic and physiological characteristics for study 1

Characteristic	Mean (SD)
N, (men and women)	60 (40 and 20)
Age, years	52.3 (5.61)
Education, years	16.9 (2.1)
BMI, kg/m^2	24.9 (4.6)
Systolic blood pressure, mm Hg	119 (12)
Diastolic blood pressure, mm Hg	72 (6)
Blood glucose, mg/dl	91.1 (10.7)
Total cholesterol, mg/dl	199.2 (35.3)
Maximal oxygen consumption, ml/kg/min	36.8 (11.8)
Waist to Hip Ratio, U	0.85 (0.08)
Serum BDNF, ng/mL	25.0 (5.9)

All the values were within the clinically normal range. Neuropsychological test scores are presented in table 2.

Table 2: Raw neuropsychological test scores for study 1

Measure	Mean (SD)
Global cognition	
Mini Mental State Examination (MMSE)	29.0 (1.2)
Weschler Test of Adult Reading (WTAR) predicted FSIQ	112.1 (6.3)
Memory	
California Verbal Learning Test- II (CVLT-II)	
Long Delay Free Recall	11.6 (3.0)
Recognition Discriminability	2.9 (0.8)
Executive Function	
Trail Making Test (TMT)	
Part A (s)	29.8 (8.6)
Part B (s)	60.0 (17.4)
Part B – Part A (s)	30.16 (15.12)
Controlled Oral Word Association (COWA) (number of words)	45.4 (10.5)
Wechsler Adult Intelligence Scale III (WAIS-III)	
Digit Span Forwards	11.3 (2.04)
Digit Span Backwards	7.4 (2.5)

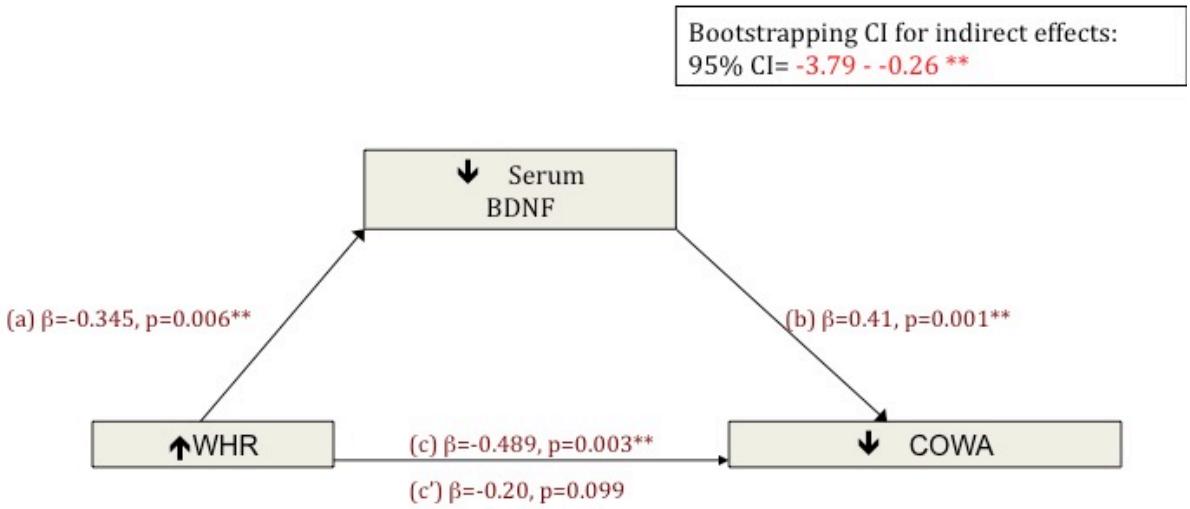
Descriptive statistics revealed a highly educated and cognitively intact sample (mean education = 16.87 years, SD = 2.1); mean estimated FSIQ = 112.11, S.D = 6.25). Participants had a mean WHR of 0.848 (S.D 0.076) and mean serum BDNF of 24.99 ng/mL (S.D 5.91).

Linear regression models examining the association between WHR and scores on each executive function neuropsychological test were completed, with age, years of education, systolic and diastolic blood pressure, blood concentrations of glucose and total cholesterol and cardiovascular fitness entered as covariates. Higher WHR was only significantly associated with poorer performance on the COWA (β=-0.489, p=0.003). As age (t=1.46, p=0.15), education (t=1.22, p=0.22), systolic blood pressure (t=1.03, p=0.31), diastolic blood pressure (t=-0.75, p=0.45), total cholesterol (t=0.34, p=0.73) and

cardiorespiratory fitness, as measured by maximal oxygen consumption (t=-0.31, p=0.76) were not significantly associated with performance on the COWA, these variables were not included in subsequent analyses. Blood glucose concentration was significantly linked with better performance on the COWA (t=2.16, p=0.036). However, since this association did not survive correction for multiple comparisons (Z, 1967), it was also excluded from subsequent analyses.

Higher WHR was significantly associated with lower levels of serum BDNF (β=-0.345, p=0.006). Lower levels of serum BDNF successfully predicted poorer scores on the COWA (β=0.41, p=0.001). The relationship between higher WHR and poorer performance on the COWA was completely attenuated when serum BDNF was included in the model (β=-0.20, p=0.099), thus fulfilling the requirements of Baron and Kenny's traditional causal steps approach for statistical mediation (Baron RM, 1986). The results of these analyses are depicted in Figure 2.

Figure 2: Multiple linear regression analyses for statistical mediation



The significance of the mediation model was further confirmed by the 95% confidence interval range (95% CI= -3.79 - -0.26) derived by Preacher and Hayes bootstrapping procedure for detecting indirect effects (Preacher *et al.*, 2004).

Discussion

To our knowledge, this is the first study to highlight BDNF as a possible mediator for the relationship between abdominal adiposity and a classic executive function task. Verbal fluency performance has been consistently linked with cardiovascular risk factors (Brady *et al.*, 2001) and in cases of post-stroke cognitive decline and vascular dementia, verbal fluency is disproportionately impaired compared with other cognitive functions such as memory (Lafosse *et al.*, 1997; Starkstein *et al.*, 1996; Wolfe *et al.*, 1990). Verbal fluency thus appears to be an area of executive functioning uniquely burdened by high cardiovascular risk.

The BDNF pathway is a plausible mechanism through which abdominal adiposity impinges upon the cognitive function. BDNF stimulates cell differentiation and proliferation by reducing inhibitor proteins and increasing the expression of neuronal nitric oxide synthase (nNOS) in adult mice (Cheng *et al.*, 2003). BDNF infusion has also resulted in neurogenesis in mouse hippocampi (Rossi *et al.*, 2006) as well as improvements in long-term memory in mice (Bekinschtein *et al.*, 2007), thus further highlighting BDNF as a credible target for intervention. However, small studies involving BDNF infusion in humans with neurologic conditions like Parkinson's Disease and Amyotrophic Lateral Sclerosis have not reported significant effects on cognition thus far (Kordower *et al.*, 2001; Ochs *et al.*, 2000). Thus, BDNF is likely to act in synergy with other disease states in humans and the interaction between BDNF and adiposity hormones likely drives the results of our study.

BDNF can also act on central nervous system functioning through N-methyl-d-aspartate (NMDA) receptors. BDNF infusion induces NMDA-dependent long-term potentiation in the insular cortices of adult mice (Escobar *et al.*, 2003). The NMDA receptor is the primary molecular device for controlling synaptic plasticity as well as a variety of neurocognitive declines, including verbal fluency (Krystal *et al.*, 1994). Thus, BDNF could have a significant effect on cortical activity by acting on NMDA receptors in the left prefrontal cortex, resulting in higher performance on verbal fluency.

A critical next step would be to determine the moderators of BDNF expression in the central nervous system in order to develop useful targeted interventions. In addition to abdominal adiposity, BDNF levels are modulated by acute and chronic stress (Murakami *et al.*, 2005). Pharmacological interventions aimed at acute stress reduction such as sertraline increased BDNF levels and promoted neurogenesis in Huntington's disease mouse models (Qi Peng, 2007). Furthermore, chronic stress is associated with higher levels of oxidative stress (Aschbacher *et al.*, 2013), which interacts with BDNF to ameliorate the deleterious cognitive effects of high fat diet in mice (Wu *et al.*, 2004). Direct examination of stress-related brain vulnerability mechanisms is beyond the scope of the current study but would be a critical next step in understanding how BDNF affects cognitive function in humans.

The main limitation of this study was the small sample size (N = 61), which limited the number and types of analyses. Larger sample sizes would allow us to examine the possible synergistic effects of stress, insulin resistance and BDNF on cognitive function via moderated mediation or mixed models. The reported effect represents the

effect of a statistical mediation procedure and may not be indicative of cause and effect. While higher levels of BDNF may be responsible for better performance on the COWA, it is also possible that both may be driven by genetic factors or developmental factors not examined in the present study. Confidence in the reported mechanism can be gained through longitudinal studies where temporal precedence between elevated BDNF levels and improvement in performance on the COWA can be clearly established. Despite the above limitations, this study is a crucial first step in directly identifying a mechanistic pathway through which central adiposity uniquely impinges upon central nervous system functioning.

STUDY 2: CENTRAL ADIPOSITY AND CORTICAL THICKNESS IN MID-LIFE

The number of individuals who have been classified as overweight or obese is increasing, currently encompassing almost 65 percent of the adult population in the United States (NCHS, 2006). Obesity has also been implicated in a host of negative health outcomes including increased risk for cardiovascular disease, gall bladder disease, cancer and overall mortality (Kopelman *et al.*, 2000). Recent evidence has shown similar deleterious effects of obesity on the brain (Gustafson *et al.*, 2004; R. Whitmer *et al.*, 2008). In particular, being classified as obese is associated with higher risk of dementia (Whitmer *et al.*, 2007) and poorer performance in cognitive tasks, particularly those that measure executive function (Stingl *et al.*, 2012). We have previously demonstrated reductions in working memory related functional activation in the brain (Gonzales *et al.*, 2010), alterations in cerebral metabolite concentrations (Gonzales *et al.*, 2012) and thinning in the cortex of the cognitive control network (Hassenstab *et al.*, 2012) as a result of obesity. Others have reported associations between obesity and increased white matter volume (Haltia *et al.*, 2007), lower grey matter density (Pannacciulli *et al.*, 2006) and lower brain volumes in patients with Alzheimer's disease and mild cognitive impairment (Ho *et al.*, 2010). Given that neurodegeneration is irreversible, it is crucial to identify the mechanisms behind this relationship early in life, so targeted preventative measures may be developed.

Adipose tissue contains metabolically active cells that could impinge upon central nervous system functioning through metabolic or hormonal pathways (Gustafson *et al.*, 2010; Gustafson *et al.*, 2007). The distribution of adipose tissue appears to selectively

impact cognitive function (Cereda *et al.*, 2007). Central adiposity is indicative of visceral fat, which is metabolically active and a more significant predictor of increased risk for cognitive decline and dementia compared to body mass index (BMI) (Cereda *et al.*, 2007; Kanaya *et al.*, 2009; Kerwin *et al.*, 2011; R. Whitmer *et al.*, 2007). Higher levels of visceral fat has been correlated with insulin resistance (Raji *et al.*, 2001), a phenomenon known to affect cerebral glucose metabolism (Doyle *et al.*, 1995) and mediate the relationship between BMI and functional activation during a working memory task (Gonzales *et al.*, 2010). Visceral fat is also independently associated with systemic inflammation in obese adults (de Luca *et al.*, 2008; Fontana *et al.*, 2007), which we have previously demonstrated to modulate concentrations of crucial cerebral metabolites (Eagan *et al.*, 2012). Furthermore, increased waist circumference/central adiposity (a proxy measure for visceral fat) has been consistently linked to poorer performance on working memory and executive control in adolescents (Schwartz *et al.*, 2013) and older adults (Wolf *et al.*, 2007). It is thus highly plausible to suggest a deleterious effect of visceral fat on neuronal integrity and cortical thickness. Nonetheless, to the authors' best knowledge, very little work has been done examining the direct effect of central adiposity on brain structure among younger, non-clinical populations. As clinically significant cognitive decline is often preceded by structural and functional indices of brain vulnerability (Dickerson *et al.*, 2009; Gonzales *et al.*, 2010), it is thus necessary to examine these associations early in life.

We have previously described a deleterious effect of higher central adiposity (as measured by waist circumference) on working memory related task activation among

middle aged adults (Gonzales *et al.*, 2014). However, research has shown that more direct measures of abdominal obesity, such as actual visceral adipose tissue mass and volume, have increased utility in predicting neurodegeneration in elderly populations (Isaac *et al.*, 2011). Higher levels of visceral fat has also been shown to correlate with lower total brain volumes in middle aged adults (Debette *et al.*, 2010), although patterns of cortical atrophy as a result of higher central adiposity in this population have not been extensively studied. Here, we aimed to expand on this previous work by examining the direct effect of visceral fat mass and volume on the structural integrity of the cortical mantle in middle-aged adults. Changes in cortical thickness have been identified in cognitively intact older adults with high BMI (Leritz *et al.*, 2011), asymptomatic amyloid positive adults (Dickerson *et al.*, 2009) and in adults with multi-domain mild cognitive impairment (Seo *et al.*, 2007). Change in thickness of the cortical mantle is thus being increasingly viewed as a marker for future cognitive decline and was chosen as the primary outcome for this study. Visceral fat was measured using dual energy X-ray absorptiometry (DXA), an X-ray scan used to accurately estimate regional fat mass and volume (Clasey *et al.*, 1999; Kaul *et al.*, 2012). This is the first study to utilize DXA technology to analyze of the effects of visceral fat on cortical thickness in healthy middle-aged adults. As we were interested in examining the early effects of visceral fat on cortical thickness, we also administered a cognitive screening battery to each participant.

Method

Participants

Adults between the ages of 40-60 years were recruited from the community through electronic and print advertisements. Individuals with a history of coronary artery disease, angina pectoris, myocardial infarctions, heart failure and cardiac surgery were excluded. Additional exclusion criteria comprised of history of neurological disease (e.g., Parkinson's disease, neurodegenerative illness, clinically significant traumatic brain injury), major co-morbid psychiatric illness (schizophrenia, anxiety), substance abuse (i.e., diagnosed abuse and/or previous hospitalization for substance abuse), metabolic disorder (thyroid disorder), smoking (within the last 2 years) or MRI contraindications. Participants who passed the initial screen were enrolled in the study after providing written consent.

Procedures

The study was approved by the University of Texas Institutional Review Board and was completed in accordance with the declaration of Helsinki, 1975. Participants underwent two separate study visits, a general health assessment where DXA data were collected and a neuropsychological/brain imaging assessment. Data were collected over a period of approximately 17 months, from August 2012 to January 2014.

General health assessment

After an 8-hour fast, blood samples were collected from the antecubital vein by venipuncture. Fasting glucose and total cholesterol level were assessed using standard enzymatic techniques. Brachial systolic and diastolic blood pressure was assessed using a

semi-automated device (VP-2000, Omron Healthcare, Bannockburn, IL, USA) after a 15-minute period of rest. Visceral fat mass and volume were estimated non-invasively via dual-energy X-ray absorptiometry (DXA) using a Lunar Dual Energy X-Ray Absorptiometry DPX (General Electric Medical Systems, Fairfield, Connecticut). This procedure required that the subject lay down on a padded table that emits energy for approximately five minutes while an arm passed overhead and involves a small amount of radiation, which is equivalent to less than 1/20 of a chest X-ray. While DXA was traditionally used to measure bone density, it has been well validated as a sensitive, relatively inexpensive tool for visceral fat measurement (Kaul *et al.*, 2012; Xia, 2014) with results comparable to that of computed tomography (CT) (Kaul *et al.*, 2012; Xia, 2014); the gold standard for measuring visceral fat.

Neuropsychological assessment

Details of the neuropsychological assessment battery administered have been described elsewhere (Gonzales *et al.*, 2014). Briefly, participants underwent a 1.5-hour battery of standard clinical instruments with established reliability and validity including the Mini Mental State Examination (MMSE), the Weschler Abbreviated Scales of Intelligence II (WASI-II), the California Verbal Learning Test-II (CVLT-II), Parts A and B of the Trail Making Test (TMT), the Controlled Oral Word Association Test (COWA), the Weschler Adult Intelligence Scale III (WAIS III) Digit Span subtest and the Stroop Color-Word Subtest (M. M. Gonzales et al., 2014). The battery was chosen to provide a comprehensive evaluation of memory, executive function and global cognitive ability.

Trained research assistants using standard administration and scoring criteria administered all assessments.

Neuroimaging

Magnetic resonance imaging was conducted using a 3T Siemens Skyra scanner equipped with a standard head coil. Anatomical scans of the entire brain were collected using high-resolution Magnetization Prepared Rapid Acquisition Gradient Echo (MPRAGE) sequences (256 × 256 matrix, flip angle = 7°, field of view (FOV) = 24 × 24 cm^2, 1 mm slice thickness, 0 gap, voxel size = 1.0 x 1.0 x 1.0 mm^3, TR = 2530.0 ms).

Neuroimaging data processing

Structural images were processed using Freesurfer Image analysis suite, which is documented and freely available for download online (http://surfer.nmr.mgh.harvard.edu). Image processing involved first motion correction and averaging of two volumetric weighted images, computerized removal of non brain tissue using a hybrid watershed/surface deformation procedure, automated Talairach transformation, intensity normalization, tessellation of the gray matter, white matter boundary, automated topology correction and surface deformation following intensity gradients to optimally place the gray/white and gray/cerebrospinal fluid borders at the location where the greatest shift in intensity defines the transition to the other tissue class. Once the cortical models are complete, a number of deformable procedures can be performed for further data processing and analysis including surface inflation, registration to a spherical atlas, which utilized individual cortical folding patterns to match cortical geometry across participants and creation of a variety of surface based

data including maps of curvature and sulcal depth. This method uses both intensity and continuity information from the entire three dimensional MR volume in segmentation and deformation procedures to produce representations of cortical thickness, calculated as closest distance from the gray/white boundary to the gray/ cerebrospinal fluid boundary at each vertex in the tessellated surface.

Statistical analysis

The procedure employed by Leritz et. al (Leritz *et al.*, 2011) was adopted here. Statistical comparisons of global data and surface maps were generated by computing a generalized linear model (GLM) of the effects of visceral fat mass and volume on thickness at each vertex in the cortical mantle using the Query Design Estimate Contrast (QDEC) interface of Freesurfer. QDEC is a single binary application included in the Freesurfer distribution that is used to perform group averaging and inference on cortical morphometric data produced by the Freesurfer processing stream. Maps were created using statistical thresholds of .05 and were smoothed to a full width half maximum (FWHM) of 20 mm. Since the QDEC analyses involved performing a GLM analysis at 160,000 vertices, maps were corrected for multiple comparisons by means of a cluster wise procedure using the Monte Carlo Null-Z simulation method adapted for cortical surface analysis and incorporated into the QDEC processing stream. For these analyses, a total of 10,000 iterations of simulation were performed for each comparison, each using a threshold of $p < .05$. This procedure has been used in similar studies examining cortical thickness (Leritz *et al.*, 2011). This method is primarily an exploratory, whole brain approach. Clinically relevant covariates such as age, blood pressure, fasting glucose level

and total cholesterol were selected based on published relationships with cortical thickness in older adults (Leritz *et al.*, 2011) and included in the model. Thickness values from significant regions of interest were extracted and loaded into SPSS version 22.0 (IBM SPSS Inc, Chicago, IL, USA) for analysis of orthogonal contrasts with cognitive test scores. Cognitive data were explored using multiple linear regression models for each of the cognitive scores with thickness in the ROI (the posterior cingulate cortex), controlling for age, years of education, systolic blood pressure, total cholesterol and blood glucose level. Adjusted Bonferroni levels of $p<0.02$, recommended for multiple intercorrelated outcomes were used as criteria for statistical significance.

Results

Descriptive statistics

One hundred and three participants were included in this study. Participants had a mean age of 49.63 years (S.D 6.45) with a mean BMI of 28.34 (S.D 5.78). Fifty-six participants identified as Caucasian, 28 identified as Hispanic/Latin American, 9 identified as African American and 10 identified as Other/did not respond. Participants had a mean visceral fat volume of 1276.33 cm^3 (S.D 1021.17 cm^3) and a mean visceral fat mass of 1206.93g (S.D 961.73g), which is consistent with other published work with similar populations (Cereda *et al.*, 2007; Kaul *et al.*, 2012; Pou *et al.*, 2007). Further information on demographic and physiologic characteristics of this sample is presented in table 3.

Table 3: Selected demographic and physiological characteristics of study 2

Characteristic	Mean (S.D)
Female, n (%)	54 (52.42)
Age, years	49.63 (6.45)
Education, years	15.99 (2.61)
BMI kg/m^2	28.34 (5.78)
Systolic blood pressure mm Hg	120.29 (13.07)
Diastolic blood pressure mm Hg	72.60 (9.46)
Blood glucose mg/dl	102.04 (36.01)
Total cholesterol mg/dl	196.67 (39.19)

This was a relatively well-educated (mean education = 15.99 years, S.D = 2.61), cognitively intact sample (mean MMSE score = 28.71, S.D = 1.39). Further information on the cognitive profile of the sample is presented in table 4.

Table 4: Raw scores on neuropsychological assessment measures for study 2

Measure	Mean score (S.D)
Global cognition	
Mini Mental State Examination (MMSE)	28.71 (1.39)
Weschler Abbreviated Scale of Intelligence- II (WASI-II)	
Vocabulary subtest	44.10 (6.09)
Matrix reasoning subtest	20.45 (4.75)
Full scale IQ	111.55 (14.81)
Memory	
California Verbal Learning Test- II (CVLT-II)	
Free recall	11.15 (2.86)
Recognition Discriminability	3.00 (0.67)
Executive Function	
Trail Making Test (TMT)	
Part A (s)	31.15 (10.72)
Part B (s)	69.23 (38.67)
Controlled Oral Word Association (COWA)	41.08 (11.31)
Weschler Adult Intelligence Scale –III (WAIS-III) Digit Span	19.94 (4.22)
Stroop color-word subtest	40.71 (10.04)

Visceral fat and cortical thickness

In order to control for non-normality of visceral fat data (Shapiro-Wilk's statistic = 0.913, $p < 0.001$ for visceral fat mass; 0.912, $p < 0.001$ for visceral fat volume), a square root transformation was carried out. Visceral fat mass and visceral fat volume were highly correlated ($r = 0.97$, $p < 0.001$). Both visceral fat mass and visceral fat volume were significantly associated with increased cortical thickness in the right posterior cingulate gyrus (X Y Z coordinates: 9, -53, -15; $t=2.38$, s.e =0.001, $p = 0.019$ and $t=2.71$, s.e =0.001, $p = 0.011$, respectively), adjusting for age, blood pressure, fasting glucose and total cholesterol. With inclusion of these covariates and stringent controls for multiple comparisons, the posterior cingulate gyrus was the only region in the entire cortical mantle that yielded results that were significant. Plots of these relationships are depicted

in figures 2 and 3. Each cm^3 increase in visceral fat volume predicted 0.31 mm higher cortical thickness. Similarly, every 1g increase in visceral fat mass predicted 0.29 mm increase in cortical thickness. As thickness of the cortical mantle in cognitively intact adults ranges between 1 and 4.5 mm (Paxinos, 1990) across the cortical mantle with an average thickness of approximately 2.5 mm (Fischl, 2000), this represents a projected increase in cortical thickness of approximately 12.4% per cm^3 of visceral fat and approximately 11.6% per gram of visceral fat.

As the posterior cingulate gyrus has been linked to poorer working memory (Sala-Llonch, 2012), we examined the associations between thickness in the right posterior cingulate gyrus and performance on the executive function tests in our screening battery. Results are presented in detail in table 5.

Table 5: Linear regression models examining the association between cognitive test scores and thickness in the posterior cingulate gyrus

Test	β	p-value
Stroop color-word (number of words in 40s)	0.14	0.127
TMT part B (time to completion)	0.17	0.071
TMT part A (time to completion)	0.06	0.532
COWA	0.03	0.744
WAIS III Digit Span total score	-0.04	0.702

While none of the associations reached statistical significance, there was a trend towards thicker cortex in the right posterior cingulate ROI being associated with poorer performance on the Trails B ($\beta = 0.17$, $p = 0.071$), a test of working memory and executive function. Bivariate correlations between visceral fat mass, visceral fat volume and performance on all other executive function measures are presented in table 6.

Table 6: Bivariate correlations between neuropsychological assessment measures and visceral fat

Measure	*r* (mass)	p	*r* (volume)	p
Global cognition				
Mini Mental State Examination (MMSE)	0.006	0.95	0.005	0.96
Weschler Abbreviated Scale of Intelligence- II (WASI-II)				
Vocabulary subtest				
Matrix reasoning subtest	-0.166	0.09	-0.156	0.11
Full scale IQ	-0.057	0.56	-0.088	0.371
Memory	-0.124	0.205	-0.136	0.165
California Verbal Learning Test- II (CVLT-II)				
Free recall	-0.252	0.009	-0.245	0.012
Recognition Discriminability	-0.090	0.360	-0.060	0.544
Executive Function				
Trail Making Test (TMT)				
Part A (s)	-0.080	0.415	-0.082	0.405
Part B (s)	-0.088	0.375	-0.078	0.427
Controlled Oral Word Association (COWA)	0.182	0.061	0.173	0.075
Weschler Adult Intelligence Scale –III (WAIS-III) Digit Span	-0.070	0.477	-0.084	0.386
Stroop color-word subtest	<0.001	0.998	0.011	0.914

Discussion

The primary finding from the current study was that higher visceral fat mass and volume were significantly associated with thicker cortex in the right posterior cingulate gyrus. This was contrary to our original hypothesis that cortical thinning will relate to abdominal obesity, but inspection of task performance was consistent with the idea that the observed relationship is indicative of potential brain/cognitive vulnerability. Previous work on obesity and brain morphology in middle age has shown reductions in total brain volume (Isaac *et al.*, 2011), increases in white matter hyperintensity volume (Debette *et al.*, 2010; Jagust *et al.*, 2005); generally indicative of vascular damage and lower hippocampal volume (Jagust *et al.*, 2005) suggestive of subcortical neurodegeneration. We have also previously reported cortical thinning in the cognitive control network among obese participants compared to successful weight loss maintainers and never obese lean participants (Hassenstab *et al.*, 2012). Here, we show selective changes in the cortical mantle in one particular region - the cortex of the posterior cingulate gyrus. Published work on functional connectivity has implicated the posterior cingulate gyrus as a key component of a network that facilitates cognitive performance during working memory tasks (Hampson *et al.*, 2006; Sala- Llonch *et al.*, 2012). Furthermore, patients with attention deficit/hyperactivity disorder have shown compromised connectivity between the posterior cingulate gyrus and other default mode network components (Castellanos *et al.*, 2008; Durston *et al.*, 2003). There have also been published studies utilizing FMRI that show significant de-activation during the Stroop color-word subtest in healthy adults (Peterson *et al.*, 1999). We have also previously shown indirect effects

of obesity on memory through elevated concentrations of cerebral metabolites in this region(Gonzales *et al.*, 2012). Taken together, the literature strongly suggests that disturbances in the structural or functional integrity of the PCC could lead to poorer performance across a number of executive function and memory tasks. While it is noteworthy that the relationship between thickness in the PCC and performance on the TMT-B in this study was only a statistical trend and performance on other executive function tests was unrelated to cortical thickness in this area, it is also important to remember that the present study included a relatively young, highly educated sample. It is not unreasonable to hypothesize that altered structural integrity of the PCC in the sample is an early marker of brain vulnerability and decline in cognitive performance would be detectable on follow-up examination. It is also important to consider that the relationship between measures of cognitive function cortical thickness across the lifespan is likely nonlinear (Schnack *et al.*, 2014) and may be moderated by genetic factors (Posthuma *et al.*, 2002). Follow up studies tracking possible cognitive decline in this population over time and examining non-linear trends and genetic influences could be a profitable future direction in this area of research.

Interestingly, a few other studies have also reported regionally specific increases in cortical thickness/brain volume as a result of metabolic risk factors, such as high serum cholesterol (Leritz *et al.*, 2011; Solomon *et al.*, 2009). Therefore the relationship, while unintuitive, may be worth further investigation.

Visceral fat is hypothesized to impinge upon the central nervous system via perturbation of insulin sensitivity in the brain (Doyle *et al.*, 1995). Mouse models have

shown that increased concentrations of uric acid that occur as a result of poor homoeostasis of insulin (Johnson *et al.*, 2007). Uric acid induces oxidative stress in adipocytes, (Sautin *et al.*, 2007) a phenomenon that results in low levels of circulating leptin (Furehwald-Schultes *et al.*, 1999; Sautin *et al.*, 2007). As leptin is protective against Alzheimer's disease associated amyloid β deposits (Fewlass *et al.*, 2004), it may be that we are seeing increases in plaque deposits in the posterior cingulate gyrus that may precede neurodegeneration. This would be consistent with published work utilizing amyloid PET imaging that link increased amyloid β burden to vascular disease in middle age (Langbaum *et al.*, 2012; Rodrigue *et al.*, 2013).While there has been research linking amyloid β burden to cortical thinning (Becker *et al.*, 2011), a complex, nonlinear relationship appears to exist between amyloid burden and brain volume in the context of dementia. Examination of the animal literature has shown higher baseline cortical thickness in amyloid precursor protein β transgenic mice compared to wild type mice in several regions including the posterior cingulate gyrus and retrosplenial cortex (Grand'Maison *et al.*, 2013). Autopsy data from the Baltimore Longitudinal Study of Aging revealed hypertrophy in the nuclei and nucleoli in the several regions of the cerebral cortex, including the posterior cingulate cortex in asymptomatic Alzheimer's Disease patients compared with cognitively intact controls and cognitively impaired subjects (Iacono *et al.*, 2008). This finding was replicated by Iacono et. al upon examination of autopsy material from the nun study (Iacono *et al.*, 2009) . Neuronal hypertrophy and astrogliosis have been suggested as possible mechanisms accounting for increases in cortical thickness in individuals at risk for cognitive impairment (Beach,

1989). Serrano-Pozo et al (2011) have described significant increases in density of astrocytes in the proximity of dense core plaques in post autopsy brains of Alzheimer's disease patients across a range of disease stages. The number of activated astrocytes also correlated significantly with number of neurofibrillary tangles in post autopsy brains at later stages of the disease process (Serrano-Pozo *et al.*, 2011). As some researchers believe that neurofibrillary tangle density contributes more strongly to disease severity and neuronal death than total plaque burden (Berg *et al.*, 1998; Giannakopoulos *et al.*, 1997) it is possible that astrogliosis as a result of increased plaque burden potentially drives increases in neurofibrillary tangles, which then leads to neurodegeneration and cortical thinning. Thus, we may speculate that visceral fat contributes to neuronal hypertrophy in the posterior cingulate cortex in middle age via increases in amyloid β deposition. Direct examination of this hypothesis is beyond the scope of this study. Larger longitudinal studies utilizing amyloid PET imaging would be needed to tease out these complex, seemingly nonlinear relationships between amyloid β deposition, cortical thickness and cognitive test performance.

The current study provides useful information on the direct impact of visceral fat on the brain in middle-aged adults. This is an adequately powered study that comprises of a cognitively intact, ethnically diverse sample. However, this study is limited by the cross sectional design, which precludes assessment of causation. A possible future direction would involve longitudinally examining this cohort, to track progression of neurodegeneration, cognitive decline and their relationships to midlife markers of peripheral metabolism. It is also possible that hereto-unexamined metabolic and genetic

factors could have moderated the effect of visceral fat on the cortical mantle; however, every effort was made to statistically control for documented relevant confounding variables such as age, blood pressure, fasting glucose and total cholesterol.

In summary, higher visceral fat volume and mass were associated with increased thickness in the right posterior cingulate gyrus. This main effect was observed over and above statistically controlling for age, systolic blood pressure, total cholesterol and glucose levels. While future research is needed to explore possible mechanisms behind this relationship, the current study is a useful first step in highlighting the complex relationship between visceral fat and structural changes to the cortical mantle in middle age.

STUDY 3: CENTRAL ADIPOSITY PREDICTS NEURONAL VIABILITY IN MID-LIFE

The prevalence of obesity has increased exponentially in the United States (NCHS, 2006) and globally (W.H.O., 2000, 2009), rendering it the fifth leading cause of mortality worldwide (W.H.O., 2009). Furthermore, obesity is implicated in a host of negative outcomes including increased risk of stroke, gall bladder disease, as well as cancer (Kopelman *et al.*, 2000). However, the association between obesity and physical health is not straightforward. In what is known as the obesity paradox, obese individuals with chronic conditions such as heart failure have lower mortality rates (Curtis *et al.*, 2005). Published research has also highlighted a similarly complex relationship between obesity and brain health. Obesity at midlife has been linked to increased risk for Alzheimer's Disease (Gustafson *et al.*, 2003), diminished working memory related functional activation (Gonzales *et al.*, 2010) and altered concentrations of crucial cerebral metabolites (Haley *et al.*, 2013). However, despite these established and well regarded findings, others have found a potential protective effect of high body mass index (BMI) and subsequent incident dementia (Qizilibash *et al.*, 2015). Still others have reported no association between midlife obesity and structural indices of brain health in old age (Albanese *et al.*, 2015). The relationship between being overweight/obese in midlife and brain health is thus complicated and warrants further investigation. Given that neurodegeneration is irreversible, it is imperative to elucidate the mechanisms that drive these changes early in life, where targeted preventative efforts may be launched.

Adipose tissue comprises of metabolically active cells that could have deleterious effects on central nervous system functioning through metabolic or hormonal pathways (Gustafson *et al.*, 2010; Gustafson *et al.*, 2007). However, the distribution of adipose tissue throughout the body appears to selectively predict cognitive ability (Cereda *et al.*, 2007). Centrally distributed adipose tissue is indicative of visceral fat, which is metabolically active and a more salient predictor of cognitive decline and dementia compared to body mass index (BMI) (Cereda *et al.*, 2007; Kaul *et a*l., 2012; Kerwin *et al.*, 2011). We have previously described the deleterious effects of various proxies of visceral fat (waist circumference and BMI) on working memory related functional brain activation (Gonzales *et al.*, 2014) and cerebral neurochemical profiles (Gonzales *et al.*, 2012) in middle aged adults. However, to our best knowledge, very little research has been done utilizing direct measures of visceral fat and its effects on brain integrity in middle age. As more direct measures of abdominal obesity have increased utility in predicting neurodegeneration in elderly populations (Isaac *et al.*, 2011), it is imperative to directly examine the impact of actual visceral fat mass and volume on brain integrity. We recently reported on the effects of visceral fat mass and volume on the thickness of the cortical mantle using an exploratory whole brain approach (Kaur *et al.*, 2015). Here, we aim to expand on this work by exploring the relationship between visceral fat and neuronal viability and metabolism measured by Magnetic Resonance Spectroscopy (MRS).

MRS allows for the identification and quantitation of several neurochemicals of neurobiological significance such as N-acetyl-aspartate (NAA), *myo*-inositol (mI) and

creatine (Cr) among others. NAA is exclusively found in the adult central nervous system (Urenjak *et al.*, 1992) and has been directly linked to changes in cognitive functioning in patients with Alzheimer's Disease (Jessen *et al.*, 2001) and neurologically intact younger adults (Grachev *et al.*, 2001). It is widely regarded as a marker of neuronal density. However, as changes in NAA are not always irreversible, it would be more accurately termed a marker of neuronal viability (De Stefano *et al.*, 1995). Cortical NAA levels have also been reported to be reduced in older adults with higher BMI (Gazdzinski *et al.*, 2010). However, very little work has been done examining the association between direct measures of visceral fat and these neurometabolites. Perturbations in levels of NAA have been associated with poorer performance on executive function tests in healthy elderly (Ross *et al.*, 2005; Valenzuela *et al.*, 2000). Furthermore, baseline cortical NAA is a useful marker for predicting post stroke cognitive decline (Ross *et al.*, 2006). *Myo*-inositol (mI), on the other hand, is an organic osmolyte and hypothesized glial marker (Brand, 1993). MI is elevated in neurodegenerative disorders such as multiple sclerosis (Fernando, 2004) and prodromal Alzheimer's Disease (Kantarci *et al.*, 2000) as well as middle-aged adults with higher BMI, indirectly impacting their memory performance (Gonzales *et al.*, 2012). Elevated levels of mI precede cognitive decline in patients with Alzheimer's Disease (Huang *et al.*, 1999), Human Immunodeficiency Virus (HIV) (Cloak *et al.*, 2004) and multiple sclerosis (Fernando *et al.*, 2004). Other neurochemical markers such as choline (Cho) are associated with white matter integrity in individuals with fragile X syndrome (Filley *et al.*, 2015).

Neurochemical markers are thus potent predictors of brain integrity and were chosen as primary outcomes for this study. As visceral fat is a more salient predictor of cognitive outcome and brain integrity than BMI, it is critical to tease out the unique contribution of high visceral fat to alterations in cerebral neurochemical profiles. Visceral adipose tissue mass and volume were directly measured using dual energy X-ray absorptiometry (DXA) (Clasey *et al.*, 1999; Kaul *et al.*, 2012).

Method

Participants

Adults between the ages of 40-60 years were recruited from the community through electronic and print advertisements. Individuals with a history of coronary artery disease, angina pectoris, myocardial infarctions, heart failure and cardiac surgery were excluded. Additional exclusionary criteria comprised of: history of neurological illness (e.g., Parkinson's disease, neurodegeneration, clinically significant traumatic brain injury), major psychiatric disorder (schizophrenia, anxiety), substance abuse (i.e., diagnosed abuse and/or previous hospitalization for substance abuse), metabolic disorder (thyroid disorder), smoking (within the last 2 years) or MRI contraindications. Participants who pass the initial screen were enrolled in the study after providing written consent. Data from individuals with uncontrolled hypertension (systolic blood pressure > 160 mm Hg), diabetes (fasting blood glucose > 126 mg/dl) and acute inflammation (fasting blood C-Reactive Protein > 10 mg/dl) were also excluded from this study.

Procedures

The study was approved by the University of Texas Institutional Review Board and was completed in accordance with the declaration of Helsinki, 1975. Participants underwent two separate study visits, a general health assessment where DXA data were collected and a neuropsychological/brain imaging assessment.

General health assessment

After an 8-hour fast, blood samples were collected from the antecubital vein by venipuncture. Fasting glucose and total cholesterol level were assessed using standard

enzymatic techniques. Brachial systolic and diastolic blood pressure were assessed using a semi-automated device (VP-2000, Omron Healthcare, Bannockburn, IL, USA) after a 15-minute period of rest. Visceral fat mass and volume were estimated non-invasively via dual-energy X-ray absorptiometry (DXA) using a Lunar Dual Energy X-Ray Absorptiometry DPX (General Electric Medical Systems, Fairfield, Connecticut). This procedure requires that the subject lay down on a padded table that emits energy for approximately five minutes while an arm passed overhead and involves a small amount of radiation, which is equivalent to less than 1/20 of a chest X-ray. While DXA was traditionally used to measure bone density, it has been well validated as a sensitive, relatively inexpensive tool for visceral fat measurement (Kaul *et al.*, 2012; Xia, 2014) with results comparable to that of computed tomography (CT) (Kaul *et al.*, 2012; Xia, 2014); the gold standard for measuring visceral fat. As visceral fat is associated with chronic systemic inflammation (de Luca, 2008; Fontana, 2007), peripheral levels of C-Reactive Protein, a marker of chronic inflammation, was measured using commercially available high sensitivity Enzyme Linked Immunosorbent Assays (ELISA) (Alpha Diagnostics, San Antonio, TX) with a minimum detectable concentration of 0.35 ng/mL.

Neuropsychological assessment

Details of the neuropsychological assessment battery administered have been described elsewhere (Gonzales *et al.*, 2014). Briefly, participants underwent a 1.5-hour battery of standard clinical instruments with established reliability and validity including the Mini Mental State Examination (MMSE), the Weschler Abbreviated Scales of Intelligence II (WASI-II), the California Verbal Learning Test-II (CVLT-II), Parts A and

B of the Trail Making Test (TMT), the Controlled Oral Word Association Test (COWA), the Weschler Adult Intelligence Scale III (WAIS III) Digit Span subtest and the Stroop Color-Word Subtest (Gonzales *et al.*, 2014). The battery was chosen to provide a comprehensive evaluation of memory, executive function and global cognitive ability. Trained research assistants administered all assessments with standard administration and scoring criteria.

Neuroimaging

Magnetic resonance imaging was conducted using a 3T Siemens Skyra scanner equipped with a standard head coil. Anatomical scans of the entire brain were collected using high-resolution Magnetization Prepared Rapid Acquisition Gradient Echo (MPRAGE) sequences (256 × 256 matrix, flip angle = 7°, field of view (FOV) = 24 × 24 cm^2, 1 mm slice thickness, 0 gap, voxel size = 1.0 x 1.0 x 1.0 mm^3, TR = 2530.0 ms) for voxel localization. Cerebral metabolite concentrations were obtained a stimulated echo sequence (svs_se_30) with the following parameters: TE/TR = 30/3000 ms, 80 excitations, 2000 Hz spectral width, volume ~6 cm^3 localized in occipitoparietal grey matter including the posterior cingulate gyrus. We concentrated on the occipitoparietal grey matter because spectroscopically detectible changes in this region correspond to severity of cognitive impairment in clinical populations (Kantarci *et al*., 2000). The commercially available LCModel software was used to quantify and identify metabolite resonances. In accordance with standard clinical quantification techniques, the concentrations of NAA, mI, choline (Cho) and glutamate (Glu) were calculated as ratios to creatine (Cr) (Bramham *et al.*, 2000).

Data analysis

All variable distributions and regression residuals were examined using the Shapiro-Wilk test for normality. The effect of visceral fat mass and volume on NAA and mI levels were assessed using simple linear regression models. Clinically relevant covariates (age, gender) were chosen a priori based on published relationships with NAA and mI (Fayed *et al.*, 2014; Zhang *et al.*, 2013) . As we have previously documented an association between mI and peripheral CRP levels (Eagan *et al.*, 2012), CRP was also included as a covariate in order to better estimate the unique contribution of visceral fat to any changes in cerebral neurochemical profiles. All statistical analyses were carried out using SPSS version 22.0 (IBM SPSS Inc, Chicago, IL, USA).

Results

Descriptive statistics

Seventy-three participants were included in this study. Participants had a mean age of 49.55 years (S.D=6.08 years) and a mean BMI of 27.88 (S.D=5.12). Forty-eight participants identified as Caucasian, 16 identified as Hispanic/Latin American, 3 identified as African American and 6 identified as Asian/Other. Participants had a mean visceral fat mass of 1022.47g (S.D=745.93g) and a mean visceral fat volume of 1083.90 cm^3 (S.D=790.67cm^3). This was a relatively well educated (mean education = 16.47 years, S.D=2.82) and cognitively intact (mean full scale intelligence quotient = 116.33, S.D= 12.78). Further information on the demographic and physiologic characteristics of this sample is presented in table 7.

Table 7: Selected demographic and physiological characteristics for study 3

Characteristic	Mean (SD)
N, (men and women)	73 (36 and 37)
Age, years	49.55 (6.08)
Education, years	16.47 (2.82)
BMI, kg/m^2	27.88 (5.12)
Systolic blood pressure, mm Hg	119 (12)
Diastolic blood pressure, mm Hg	72 (6)
Blood glucose, mg/dl	91.1 (10.7)
Total cholesterol, mg/dl	199.2 (35.3)
Maximal oxygen consumption, ml/kg/min	36.8 (11.8)
Waist to Hip Ratio, U	0.85 (0.08)
Serum C-Reactive Protein mg/dL	3.02 (2.61)

Visceral fat and cerebral neurochemical profiles

In order to control for non-normality of visceral fat data (Shapiro-Wilk's statistic = 0.936, p <0.001 for visceral fat mass; 0.936, p <0.001 for visceral fat volume), a square root transformation was carried out.

Both visceral fat mass and volume were significantly associated with reduced concentrations of NAA/Cr in the posterior cingulate gyrus, controlling for age, gender and chronic inflammation (β = -0.29, p=0.03, R^2 = 0.22 for visceral fat mass and β = -0.28, p=0.04, R^2 = 0.22 for visceral fat volume).

After controlling for the effects of age, gender and chronic inflammation, visceral fat mass and volume were also related to significant increases in the mI/Cr ratio in the posterior cingulate gyrus (β = 0.36, p=0.01, R^2 = 0.15 for visceral fat mass and β = 0.36, p=0.01, R^2 = 0.15 for visceral fat volume).

Visceral fat mass and volume were not related to Cho/Cr or Glu/Cr after controlling for age, gender and chronic inflammation (ps>0.05).

Discussion

To our knowledge, this is the first study to utilize a direct measure of visceral fat to examine the effects of visceral adipose tissue on brain integrity as measured by cerebral neurochemical profiles. Our results point to concentrations of NAA/Cr and mI/Cr in the posterior cingulate gyrus as particularly vulnerable to the effects of high visceral fat in middle aged adults. Visceral fat mass and volume was associated with lower levels of NAA/Cr and elevated levels of mI/Cr in the posterior cingulate gyrus. These effects remained significant after controlling for age, gender and systemic inflammation.

A particularly interesting finding was the relationship between NAA/Cr levels and high visceral fat. Mouse models of Alzheimer's disease suggest that elevations in cerebral concentrations of mI could precede reductions in concentrations of NAA (Chen *et al.*, 2010). It is however, possible that NAA levels in our sample are moderated by other factors associated with obesity. For example, NAA levels could be modulated by levels of cerebral Brain Derived Neurotrophic Factor, a key neurotrophin responsible for synaptic plasticity and neuronal regeneration (Bramham CR, 2005; M. Mattson, Maudsley, S, Martin B, 2004) that is diminished in mouse models of obesity (Kernie *et al.*, 2000). Recently published work has revealed an association between genetic risk for low BDNF and lower hippocampal NAA levels (Egan *et al.*, 2003; Stern *et al.*, 2008). Furthermore, examination of the animal literature have revealed simultaneous increases in cortical NAA levels and reductions in cortical mI levels after intraventricular BDNF infusion (Zhang *et al.*, 2013). The BDNF pathway is thus a mechanism that warrants

further investigation. Direct examination of a possible visceral fat-BDNF-neurochemistry relationship is, however, beyond the scope of the current study.

We also demonstrate elevations in mI in individuals with high visceral fat. Previously, we have highlighted an indirect effect of elevated BMI on memory performance through elevations in mI (Gonzales *et al.*, 2012). This is consistent with other published work on mI and cognition in multiple disease states (Cloak *et al.*, 2004; Fernando *et al.*, 2004; Huang *et al.*, 1999). It has been hypothesized that changes in mI occur in the context of systemic inflammation (Eagan *et al.*, 2012). Our results show an effect of visceral fat on mI/Cre concentrations over and above that of inflammation, however, that warrants further investigation.

Visceral fat could perturb cerebral mI/Cre concentrations through increasing blood brain barrier (BBB) permeability. The BBB consists primarily of endothelial cells that are joined by endothelial tight junctions (Pan *et al.*, 2007). Adiponectin, an adipokine that is markedly reduced in individuals with high visceral fat (Warren *et al.*, 2012), has been shown to stimulate production of nitric oxide (NO), a critical vasodilator in endothelial cells (Chen *et al.*, 2003). Furthermore, adiponectin modifies the deleterious effects of pro-inflammatory cytokines on endothelial cells that comprise the BBB (Spranger *et al.*, 2006).

Compromised integrity of the BBB could lead to elevated mI levels in cerebral glial cells, which could in turn, lead to proliferation of astrocytes (Hattingen *et al.*, 2007). Serrano-Pozo et al (2011) have described significant increases in density of astrocytes in the proximity of dense core plaques in post autopsy brains of Alzheimer's disease

patients across a range of disease stages. The number of activated astrocytes also correlated significantly with number of neurofibrillary tangles in post autopsy brains at later stages of the disease process (Serrano-Pozo *et al.*, 2011). Elevations in mI could thus be critical early markers of a neurodegenerative process.

It is noteworthy that we did not find significant relationships between visceral fat and other neurochemical markers (Glu/Cr and Cho/Cr). This is unsurprising, given the stronger body of research highlighting the effects of NAA/Cr and mI/Cr on cognition across disease states. Cho/Cr and Glu/Cr are reduced in individuals with Fragile X Syndrome (Bruno *et al.*, 2013; Filley *et al.*, 2015). It is thus possible that levels of Cho/Cr and Glu/Cr perturbed by genetic influences unrelated to obesity.

The main limitation of this study lies in the cross-sectional design, which negates determination of causality. However, we demonstrate a relationship between measures of visceral fat and neurochemical markers that have been highlighted in the literature as early risk factors for cognitive decline and Alzheimer's Disease. Our sample size (n =73), while larger than other published MRS research (Gazdzinski *et al.*, 2010), was modest and thus limited the number of analyses that were possible. Future research should include mediation/moderation models that highlight possible interactive effects of neurochemistry, as well as other potential risk factors known to affect cognitive decline such as genetic risk, stress, diet and exercise.

Despite the above limitations, the current study is a useful early step in teasing out the mechanisms behind the complex relationship between obesity and cognition. As the

sample comprised of middle aged, cognitively intact individuals, it is provides crucial information on preclinical effects of visceral fat that precede neurodegeneration.

SYNTHESIS

The overall goal of this project was to explore the relationship between visceral fat and brain vulnerability in middle age. The three studies focused on different indices of brain vulnerability; specifically cognitive function (study 1), structural integrity (study 2) and neuronal viability (study 3). Study 1 tested a basic biological pathway with serum BDNF as a mediator for the effects of a proxy measure for visceral fat (hip-to-waist ratio) and cognitive function, while studies 2 and 3 employed increasingly sophisticated methodology such as DXA, structural MRI and ^{1}H- MRS to directly examine the effects of visceral fat on the brain in middle age. In closing, I will present revisit the main findings and comment on future directions including implications for early intervention.

Visceral fat and cognitive function: mediation by serum BDNF

Study 1 examined the possible role of BDNF as a mediator of the relationship between central adiposity and executive function. When examining the data presented in Studies 2 and 3, there was a trend towards a significant relationship between visceral fat and poorer performance on the COWA, above the effect of age (β=-0.13, p =0.06). In study 1, we found that serum BDNF completely statistically mediated the documented relationship between central adiposity (as measured by waist to hip ratio) and performance on an executive function task (verbal fluency). This statistical mediation was replicated when we re-ran the analyses in an integrated dataset combining data from studies 1, 2 and 3. FMRI research on cognitively intact young adults has revealed significant deactivation in the posterior cingulate gyrus during a verbal fluency task (Schlosser, 1998). Interestingly, study 2 reported changes in cortical thickness the same

region among middle-aged adults with high visceral fat (Kaur *et al.,* 2015). Verbal fluency performance has also been consistently linked with cardiovascular risk factors (Brady *et al.*, 2001) and in cases of post-stroke cognitive decline and vascular dementia, verbal fluency is disproportionately impaired compared with other cognitive functions such as memory (Lafosse *et al.*, 1997; Starkstein *et al.*, 1996; Wolfe *et al.*, 1990). Verbal fluency thus appears to be an area of executive functioning uniquely burdened by high cardiovascular risk. Individuals with high visceral fat also tend to have significantly higher risk of cardiovascular events such as coronary heart attacks, stroke and mortality caused by cardiovascular events (Kannel *et al.*, 1991; Ritchie *et al.*, 2007). It is thus possible that high central adiposity drives cardiovascular disease induced declines in verbal fluency that lead to more generalized cognitive impairment. Direct examination of this is, however, beyond the scope of this study.

One potential mechanism through which visceral fat induced reductions in BDNF could impinge upon central nervous system functioning is through disruption of the insulin regulation pathway. Insulin resistance is strongly associated with reduced BDNF (Levinger *et al.*, 2008) and infusion of BDNF into the lateral ventricles of diabetic mice normalizes glucose regulation (Nakagawa *et al.*, 2002). As insulin levels became available for our set of participants, follow-up examination of the data revealed that while insulin sensitivity was significantly related to higher WHR in our data set, it was not related to BDNF levels or performance on the COWA in our participants. These results were somewhat puzzling; yet, BDNF expression in the brain is extremely complex and influenced by multiple factors. Therefore, the BDNF-insulin resistance relationship in our

set of participants could have been moderated by another metabolite of neurobiological significance. Serotonin, for example, has been highlighted as a biomarker that stimulates the expression of BDNF, thus regulating synaptic plasticity and contributing to insulin resistance syndrome (Mattson *et al*, 2004). The relationship between BDNF, insulin regulation and the central nervous system is thus complex and requires further examination. As the correlation between insulin resistance and visceral fat is well documented (Raji *et al.*, 2001) and BDNF has a key role in the pathogenesis of insulin resistance syndrome (Duan *et al*, 2003; Duan *et al.*, 2001; Nakagawa *et al.*, 2002), examining this relationship would be critical in enhancing our understanding of the nuanced effects of visceral fat on the brain.

Visceral fat and brain vulnerability

Study 2 revealed a significant positive relationship between visceral fat and cortical thickness in the posterior cingulate gyrus among cognitively intact, middle aged adults. While this was contrary to our initial hypothesis that high visceral fat would lead to cortical thinning, other published studies have also reported regionally specific increases in cortical thickness/brain volume as a result of metabolic risk factors, such as high serum cholesterol (Leritz *et al.*, 2011; Solomon *et al.*, 2009). This may point to a more complex relationship between metabolic risk factors such as visceral fat and structural integrity of the brain that warrants further investigation.

Visceral fat induced changes in cortical thickness could occur in the context of systemic inflammation. Visceral fat has been linked to increased secretion of pro-inflammatory cytokines such as Interleukin 6 (IL-6), C-Reactive Protein (CRP) and Tumor Necrosis

Factor-alpha (TNF-α)(Fontana *et al.*, 2007; Malavazos *et al.*, 2007) through disruption of insulin regulation mechanisms in the central nervous system (de Luca *et al.*, 2008). Closer examination of data from study 2 confirms this; CRP was significantly related to higher visceral fat ($r = 0.18$, $p < 0.01$) and insulin sensitivity ($r = -0.24$, $p < 0.01$). There was also a non-significant trend where higher CRP was correlated with higher thickness in the posterior cingulate cortex ($r = 0.21$, $p = 0.07$). CRP and its mediating cytokines are capable of crossing the blood brain barrier, where they can propagate an inflammatory response (Banks *et al.*, 1995). Higher levels of systemic neuroinflammation are also seen very early in plaque development (Apelt *et al.*, 2001). Furthermore, anti TNF-α treatment reduces amyloid plaques and tau phosphorylation in transgenic mice (Alpine *et al.*, 2009; Mattson *et al.*, 1997; Shi *et al.*, 2010). Taken together, the literature supports a hypothesis that neuropathological deposits in the cerebral cortex could occur as a result of high visceral fat and would precede neurodegeneration. However, these proposed mechanisms are speculative at this point and require validation from future studies directly assessing these components. Amyloid PET, for example, would be useful to examine potential early plaque build up in the posterior cingulate gyrus that could account for increases in cortical thickness as seen on structural MRI.

Study 3 highlights reductions in concentrations of NAA and increases in concentrations of mI in the posterior cingulate gyrus among middle-aged adults with high visceral fat over and above the effects of systemic inflammation. As NAA and mI concentrations are significantly linked to cerebral BDNF levels (Zhang *et al*., 2013), it is possible that concentrations of these metabolites are perturbed by visceral fat induced

reductions in BDNF. BDNF can act on central nervous system functioning through N-methyl-d-aspartate (NMDA) receptors. BDNF infusion induces NMDA-dependent long-term potentiation in the insular cortices of adult mice (Escobar *et al.*, 2003). The NMDA receptor is the primary molecular device for controlling synaptic plasticity as well as a variety of neurocognitive declines (Krystal *et al.*, 1994). Infusion of ketamine, an NMDA antagonist, is associated with reductions in fMRI activation in the posterior cingulate gyrus among cognitively intact younger adults (Northoff *et al.*, 2005). Published work has also revealed reductions in thalamic NAA among chronic ketamine users relative to age matched controls (Stone *et al.*, 2014). Thus, BDNF could have a significant effect on cortical neurochemistry by acting on NMDA receptors in the posterior cingulate gyrus. In our sample, BDNF was not significantly related to concentrations of NAA/Cre and mI/Cre. This could be attributed to the fact that participants in this study had much lower BDNF overall compared to participants in study 1, where many of the participants were specifically recruited from a pool of endurance trained adults. In general, likely due to the differences in the baseline characteristics of the two samples, the range of BDNF values was greater in study 1 as compared to studies 2 and 3 (3.8 ng/mL to 37.2 ng/mL vs 0.1 ng/mL to 6.1 ng/mL). Additionally, other lifestyle factors can also suppress BDNF expression. Examination of the animal literature indicates that sleep deprivation can reduce BDNF mRNA expression in the neocortex and hippocampi of adult mice (Guzman-Marin *et al*, 2006). As the participants in studies 2 and 3 had much higher BMIs and lower fitness levels than the participants in study 1, it is not unreasonable to hypothesize that the prevalence of sleep problems among individuals in studies 2 and 3

could have been higher; thus, explaining some of the variance in BDNF levels. It would hence be necessary to examine these factors to determine their exact effects on our sample.

Limitations

It is pertinent to note that all three studies included only cognitively intact middle-aged adults without any neurological illness. An important limitation across all 3 studies was a lack of controls for medication use. However, as most cardiovascular risk factors that could potentially influence the results were considered carefully and included as covariates in regression models where appropriate, it is possible to be reasonably confident in the results reported. Furthermore, only 15.5 % of all participants were currently prescribed any form of heart, cholesterol, blood pressure medications or steroids. Since many of those conditions go undiagnosed and unmedicated at midlife, we judged it more important to control for the actual levels of blood pressure, blood glucose and lipids. In addition, we were only able to obtain serum BDNF from the periphery. BDNF in the brain might have a stronger effect on structure and neurochemistry than peripheral BDNF. All studies also included people with high IQ (mean IQ = 112.1 for Study 1, 111.55 for Study 2, 116.33 for Study 3). It can thus be posited that the changes in brain structure and function highlighted here provide evidence of a worrying trend of preclinical neurodegeneration despite high cognitive reserve.

Moving forward

As discussed in the introduction, this project is a critical first step in improving our general understanding of the impact of visceral fat on brain structure and function in

middle age. All the studies highlighted changes in brain structure, chemistry and function in individuals with high visceral fat. Statistical power considerations and the cross sectional nature of the data limited the types of analyses that could be performed.

A natural progression of these studies would be to examine the effects of possible modifiable risk factors that could account for the effect of visceral fat on brain structure and function. Psychosocial stress, for example, is known to affect levels of cerebral BDNF (Murakami S, 2005), which could in turn modulate concentrations of NAA as well as changes in brain structure. Stress induction has also been connected with reduced BDNF mRNA coding in mouse hippocampi (Smith *et al.*, 1995). It would thus be of interest to examine the effects of stress reduction as an adjuvant therapy to prevent cognitive decline.

Exercise (Oliff *et al.*, 1998) and dietary restriction (Duan *et al.*, 2001) also improve BDNF regulation and may ameliorate some of the negative effects of visceral fat on the brain. If systemic inflammation modulates these relationships further, it might be pertinent to examine if non-steroidal anti-inflammatory drugs (NSAIDs) would be useful in mitigating the deleterious effects of visceral fat on the brain. As mentioned previously, the irreversible nature of neurodegeneration renders research on successful preventative measures a public health imperative.

In addition, the possible moderating effects of other psycho-social variables that were not included in these analyses for statistical power concerns should be further examined. Socio-economic status (SES), for example, is associated with significantly increased risk of developing incident dementia in late life (Karp *et al.*, 2004). In

particular, when education is used as a marker of low SES, SES is associated with a significantly higher relative risk of developing incident dementia (Karp *et al.*, 2004, Evans *et al*, 1997). As all three studies included relatively highly educated individuals (mean education =16.9 years for study one, 15.99 years for study 2 and 16.47 years for study 3), it would be necessary to examine if these effects are replicated in individuals with lower education/SES.

Race is another psychosocial variable that could potentially have effects on the relationship between visceral fat and central nervous system functioning. There is evidence for lower levels of visceral fat in middle aged African Americans compared with Caucasians and Hispanics with similar BMI and waist circumference (Carroll *et. al*, 2008). Furthermore, despite lower levels of visceral fat, African Americans have similar or higher levels of inflammatory biomarkers, such as CRP that are commonly associated with high visceral fat (Carroll *et. al*, 2009). It is thus possible that race based genetic differences may moderate the effects of visceral fat on central nervous system functioning. Direct examination of this was, however, beyond the scope of this study due to statistical power considerations.

In addition to the above, it would be interesting to examine the relationship between visceral fat and brain vulnerability earlier in the life span. There is strong evidence for obesity-driven changes in executive function in children and adolescents (Lokken *et. al,* 2009. Verdejo-Garcia *et. al*, 2010). In particular performance on tests that involve mental flexibility (which is also assessed in verbal fluency tasks) appear to be selectively impacted by high BMI (Lokken *et. al,* 2009. Verdejo-Garcia *et. al*, 2010). The

direction of this relationship between obesity and executive function in childhood has, however, been debated (Smith *et. al*, 2011). Specifically, it has been hypothesized that poorer executive function in childhood leads to disinhibited eating, which could in turn, promote childhood obesity (Maayan *et. al*, 2011). To the best of my knowledge, there has however been no research thus far on the selective impact of visceral fat in childhood and adolescents. Evidence for this would necessitate the development of interventions early in life, before the onset of neurodegenerative processes.

Summary

In summary, all three studies appear to emphasize a possible detrimental effect of high visceral fat on brain structure and function in middle age. This project provided new evidence directly examining the effect of visceral fat on established indices of brain structure (cortical thickness), function (cognitive performance) and vulnerability (concentrations of cerebral metabolites) among cognitively intact middle aged adults. In addition, we provided preliminary evidence for a possible mechanistic pathway explaining this relationship by highlighting circulating BDNF as a potential mediator. Future directions include examining interactive effects of other biomarkers and directly examining treatment options both in middle age and earlier in the life span.

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