RETINAL MOPHOLOGY AND INTELLIGENCE

BY

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THESIS

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ABSTRACT

Objective: To investigate the relationship between retinal morphometric measures and intellectual abilities among adults with overweight and obesity.

Methods: Adults between 25-45 years (*N*=55, 38 females) with overweight or obesity (BMI ≥25.0 kg/m^2) underwent an optical coherence tomography (OCT) scan to assess retinal nerve fiber layer (RNFL) volume, ganglion cell layer (GCL) volume, total macular volume, and central foveal thickness. Dual-Energy X-ray Absorptiometry was used to assess whole-body adiposity (%Fat). The Kaufman Brief Intelligence Test-2 was used to assess general intelligence (IQ), fluid, and crystallized intelligence. Hierarchical linear regression analyses were performed to examine relationships between adiposity and intelligence measures following adjustment of relevant demographic characteristics and degree of adiposity.

Results: Although initial bivariate correlations indicated that %Fat was inversely related to fluid intelligence, this relationship was mitigated by inclusion of other demographic factors, including age, sex, and education level. Regression analyses for primary outcomes revealed that RNFL was positively related to IQ and fluid intelligence. However, only GCL was positively related to crystallized intelligence.

Conclusion: This work represents the first study to demonstrate that specific retinal morphometric measures – assessed using OCT – can be utilized to study intellectual abilities among adults with overweight and obesity.

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TABLE OF CONTENTS

CHAPTER 1: INTRODUCTION

Obesity prevalence is a growing global public health issue (Swinburn, Sacks, Hall, et al., 2011). In 2015, there were 604 million adults with obesity worldwide, representing a greater than two-fold increase in prevalence since the 1980's (Afshin, et al., 2017). In the United States, obesity is estimated to affect approximately 40% of the adult population (Hales, et al., 2017). Excess fat mass or adiposity is known to directly contribute to a wide range of metabolic disorders and chronic diseases including type 2 diabetes and cardiovascular disease (Malnick & Knobler, 2006). However, overweight and obesity are also related to mood disorders including anxiety and depression and increasing evidence suggests that the detrimental consequences of obesity also extend to cognitive function and brain health (Romain, Marleu, & Baillot, 2018) including greater risk for dementia in older age (Luchsinge & Gustafson, 2009; Gustafson, 2006).

While the underlying mechanisms remain unclear, evidence from magnetic resonance imaging (MRI) studies indicates that obesity is predictive of variations in brain structure and function that often accompany cognitive deficits including reduced synaptic plasticity (Erion, et al., 2014), reduced processing speed (Sanz, Ruidavets, & Bongard, 2012), and lower gray matter volume (Walther, et al., 2009). Population-based studies have revealed that, akin to aging, increasing Body Mass Index (BMI) is longitudinally associated with declining gray matter volume (13 to 16% reduction per unit increase in BMI) in the temporal lobe (Gustafon, et al., 2004). Similarly, obesity has also been linked to MRI measures of white matter including hyperintensities (Jagust, Harvey, & Mangus, 2005; Gustafson, Steen, & Stoog, 2004; Stanek, et al., 2011) . Therefore, conventional neuroimaging techniques, primarily MRI, have revealed links between gray matter and white matter outcomes and obesity. However, the use of MRI presents many practical challenges including high financial costs, contraindications, susceptibility to movement

artifacts, technical expertise necessary for scan acquisition and analyses, and limited mobility or accessibility for populations. Therefore, there is increasing need for determining the efficacy of alternative neuroimaging techniques with the requisite sensitivity to cognitive abilities and brain health, particularly among individuals with overweight or obesity.

Recent evidence indicates the morphometric measures of the human retina, studied using optical coherence tomography (OCT), have the potential to be utilized as markers of gray and white matter in the brain (Mutlu, et al, 2017). Since the human retina is formed embryonically from neural tissue and is integrated into the neural system via the optic nerve, it is possible that structural abnormalities in brain tissue may be reflected in the retina (Mutlu, et al, 2017; Chang, et al., 2014). Additionally, imaging the retina, as proxy for brain, provides unique advantages since it can be visualized noninvasively at the cellular level due to its transparent nature, allowing for inexpensive testing of neurological biomarkers in clinical settings (Chang, et al., 2014). OCT is a 3-dimensional retinal imaging technique that relies on low-coherence near infrared interferometry (Huang, et al., 1991) to segment the various structural components of the retina including, but not limited to, the retinal nerve fiber layer (RNFL), ganglion cell layer (GCL), and macular volume and thickness. Although OCT is often used in clinical settings to detect abnormalities in the eye and monitor the progression of ocular diseases, retinal neurodegeneration has been recently correlated with cerebral atrophy suggesting that neuronal damage may occur simultaneously in the retina and throughout the brain (Ong, et al., 2015). Additionally, the thickness of different layers of the retina are related to specific brain subcomponents of brain matter. For example, RNFL is composed of axons and RNFL thickness has been related to cerebral white matter. On the other hand, neuronal cell bodies comprise the GCL and may be reflective of cerebral gray matter (Mutlu, et al, 2017). The RNFL relationship to white matter has received further support from studies

among patients with Multiple Sclerosis demonstrating that RNFL is correlated with white matter tracts that are functionally separated from the visual system (Scheel et al., 2014). Several studies involving adults with Alzheimer's have shown that these patients have reduced RNFL and GCL (Cheung et al., 2015; Thomson, et al., 2015). Interestingly, thinner RNFL and GCL have also been associated with smaller temporal lobe structures including the hippocampus which is vital for memory and learning across the lifespan (Mutlu, et al, 2017). Although emerging evidence points to the utility of OCT as a neuroimaging technique, data relating retinal morphometric measures and cognitive function remain limited. Pertinent to the work presented in the current study, the extent to which different retinal layers correspond to aspects of intellectual abilities among individuals with overweight and obesity has not been directly examined.

Intelligence represents a critical cognitive ability known to support vital cognitive processes such as executive function and the acquisition of knowledge and learning (Colom, et al., 2010). Intelligence can be conceptualized as general intelligence (i.e., intelligence quotient [IQ]) or its separable components of crystallized intelligence and fluid intelligence. Studying specific constructs of intelligence is important given that fluid and crystallized intelligence exhibit differential susceptibility to factors such as aging (Craik & Bialystok, 2006; Park & Reuter-Lorenz, 2009). Crystallized intelligence reflects the ability to use previously acquired knowledge and is therefore amenable to learning while fluid intelligence is thought to represent the ability to adapt to new situations (Cattell, 1963). In the context of obesity, studying these different measures of intelligence may provide insights into components of cognitive function that exhibit sensitivity to obesity-related cognitive impairments. However, to our knowledge, the relationship between retinal morphometric measures and intellectual ability among adults with overweight and obesity has not been previously studied.

Accordingly, the present work aimed to utilize OCT to assess the relationship between retinal morphometric measures and different constructs of intelligence among adults with overweight or obesity. Given prior evidence indicating that thicker RNFL and GCL are related to greater gray matter and white matter volumes among older adults, we hypothesized lower thickness in RNFL and GCL will be associated with poorer performance across all measures of intelligence (i.e. IQ, fluid, and crystallized).

1.1. PURPOSE

The purpose of the proposed study was to investigate the relationship between retinal measures and intelligence in healthy adults with overweight or obesity.

1.2. HYPOTHESES

It was hypothesized that specific layers of the retina would be correlated to general intelligence, fluid intelligence and crystallized, specifically the Ganglion Cell Layer (GCL) and the Retinal Nerve Fiber Layer (RNFL).

CHAPTER 2: LITERATURE REVIEW

2.1. OBESITY

The prevalence of obesity, defined a body mass index (BMI) ≥30kg/m^2, continues to be a public health concern in the United States with 39% of adults classified as obese in 2015-2016 (Hales, et al., 2017). Obesity has over 20 co-morbidities, including diabetes mellitus, hypertension, coronary heart disease, high serum cholesterol, increased risk for certain cancers, sleep apnea, osteoarthritis, and stroke (Kopelman, 2000; Ogden, et al., 2010; Ebbeling, Pawlek, Ludwig, 2002), making obesity one of the leading causes of preventable deaths (Biro, 2010). Additionally, as obesity increases the disease burden of obesity increases simultaneously (Paeratakul, et al., 2002). Furthermore, obesity has been shown to reduce life expectancy by up to nine years in Caucasians and six years in African-Americans (Fontaine, et al., 2003). BMI is the most widely used classification system for measuring obesity which is a height to weight ratio (kg/m^2) and therefore is a convenient and non-invasive way to assess the risk of obesity. However, since BMI is only a ratio, it does not adequately characterize body composition including bone mass, lean tissue mass, and adiposity. Alternatively, Dual-energy X-ray absorptiometry (DXA) can accurately measure bone mass, lean tissue mass, and fat mass (Flegal, 2010). Fat, bone mineral, and lean tissue are analyzed using low- and high-energy photons and the amount of each composition is measured on a pixel basis. Currently, there is no widely accepted clinical obesity ranges of body fat percentages. However, Kelly, Wilson, and Heymsfield (2009) analyzed n=9304 (female n=4666) DXA scans from the 1999-2004 NHANES research, with participants starting at age of 8 and continuing along the life span to 85 years and over. Utilizing the BMI classification system - underweight <18.5 kg/m^2, normal weight 18.5-24.9 kg/m^2,

obesity class I 30.0-34.9 kg/m^2, obesity class II 35.0-39.9 kg/m^2, and obesity class III ≥40.0 kg/m^2 - similar thresholds were generated for Fat Mass Index (FMI; fat mass/height^2). They found that in adult males > 6 FMI and in adult females > 9 FMI related to the BMI classifications of overweight and obesity. Since this measure is gender specific, lean tissue does not impact the classification. More research will need to be conducted to have a widely accepted body fat percentage range for adults.

One of the co-morbidities of obesity is diabetes mellitus, which can have additional complications including diabetes retinopathy which is a leading cause of blindness in adults (Barber, 2003). In the Wisconsin Epidemiologic Study of Diabetic Retinopathy, up to 21% of diabetic patients with type 2 diabetes had Diabetic Retinopathy at the time of diabetes diagnosis and most patients developed retinopathy throughout disease progression. Additionally, throughout the study 1.6% of these patients were legally blind (Fong, et al., 2004). Another complication that may come from diabetes mellitus is the increased risk of dementia (Biessels, et al., 2006) and other factors of brain health.

Research has found that the impact of obesity may reach beyond metabolic diseases and impact brain health. Increasing total body fat appears to be related to deterioration of cognitive function (Naderali, Ratcliffe, & Dale, 2009). Insulin resistance is another co-morbidity of obesity (Lloyd, Langley-Evan, & McMullen, 2010), and insulin plays a significant role in synaptic plasticity and memory (Watson & Craft, 2004). This may be due to the central nervous system's role in insulin receptor signaling. Decreased insulin receptors in the brain has been associated with degenerative dementia (Zhao et al., 2004). In a prospective study of 2,798 adults, without dementia, those with a BMI > 30 kg/m^2 were at a higher risk of developing dementia five years later compared to those with a normal

weight BMI of 20-25 kg/m^2 (Fitzpatrick et al., 2009). Interestingly, Miller and colleagues (2006), found that participants with non-early onset morbid obesity (n=18) performed significantly lower in their General Intelligence Ability and their total achievement tests compared to their Early onset morbid obesity (EMO) siblings (n=21). Within the context of this study, EMO was defined as participants who exceeded 150% of their Ideal Body Weight or had a BMI of greater than 97% before the age of 4 years. Additionally, MRI results showed that five of the EMO participants had white matter lesions while no control participants had lesions (Miller, et al., 2006). Obesity's impact on white matter was further supported by Stanek et al., (2011) (n=103), who found that adults with a BMI of 30 or greater had significantly reduced white matter integrity. Obesity has been related to cognitive deficits throughout multiple studies (Smith, et al., 2011, Prickett, Brannan, & Stolwyk, 2015) starting in childhood (Khan, et al., 2015; Li, et al., 2008) and continuing through older adulthood (Nguyen, et al., 2014). Importantly, obesity has also been associated with deficits in prefrontal (Kamijo, et al. 2012), orbitofrontal cortexes (Reinert, Po'e, & Barkin, 2013) reduced synaptic plasticity (Erion et al., 2014), and lower grey matter volume (Walther et al., 2009). Additionally, in animal models, diet-induced obesity has also been shown to have a relationship with retinal degeneration (Marcal et al., 2012).

2.2. RETINAL MORPHOLOGY

The retina is developed embryonically along with other neural tissues and is connected to the brain by the optic nerve. OCT advancements since the 1990's have enabled clinicians to assess neurodegeneration in the retina noninvasively with biopsy-like precision (London, Behnhar, & Schwartz, 2012; Mutlu, 2017). While OCT is a comparatively recent technological innovation, the equipment and software has made significant advancements in

the previous decade. In 1991, OCT used 2-dimensional cross-sectional images of the retina using Time-domain. However, in 2004, OCT began to use 3-dimensional images using Spectral-domain (SD-OCT, also known as Fourier domain OCT) (Yanni, et al., 2013). The SD-OCT added ultrahigh speed imaging to the Time-Domain's ultrahigh resolution (Yi, Chen & de Boer, 2006). The Time-Domain OCT required a physically moving reference mirror, while SD-OCT utilized a reference beam and sample beam to assess the spectral interference pattern obtained from a spectrometer and an array detector (Neto & Rebhun, 2018). With these advancements, the SD-OCT images are higher quality, with a resolution of 5 μm, than the Time-Domain OCT and acquisition of the images was faster with SD-OCT taking 20,000 scans per second, while the Time-Domain OCT was only capable of 500 scans per second. (Pierro & Gagliardi, 2014).

The retina has ten layers (**Figure 1**; Know Your Retinal Layers, 2016) , the internal limiting membrane, retinal nerve fiber layer (RNFL), ganglion cell layer (GCL), inner plexiform layer (IPL), inner nuclear layer, outer plexiform layer, Henle fiber layer, outer nuclear layer, external limiting membrane, photoreceptor layers. Each layer is visible through the OCT by segmenting the layers according to their edges with the OCT software. The inner three layers of the retina, RNFL, GCL, and IPL, can be grouped into the Ganglion Cell Complex which represents 30% of retinal thickness. The RNFL contains the axons of the Retinal Ganglion Cells (RGC), the GCL is composed of the bodies of the RGCs, and the IPL is the dendrites of the RGCs. The RGCs are important to visual function as they send neural messages from all visual information to optic nerve which connects with the central nervous system (Skalicky, 2016). The RNFL has been heavily studied in glaucoma research as the decreasing in RNFL thickness correlate with the loss of the RGC axon bundles (Hoyt

& Newman, 1972; Hoyt, Frisen, & Newman, 1973; Sommer et al., 1991). The IPL is the beginning of several functions in the visual system, motion detection, contrast, hue, and brightness (Remington, 2012).

With the advances in OCT, clinicians can utilize this imaging technique to assess disease progression of the clinical macula which is the 3.45 mm circle surrounding the fovea. The center of the clinical macula is the fovea, which is a depression which has no vessels. Without the vessels, the fovea has minimal light scattering allowing for high visual acuity (Dubis, et al., 2012). Surrounding the fovea, is the parafovea which has the thickest GCL, IPL, and inner nuclear layers. These layers are responsible for processing signals from both foveal and parafoveal photoreceptors (Knighton & Gregori, 2012). The parafovea is surrounded by the perifovea, which is primarily composed of the axons of the photoreceptors (Skalicky, 2016). Unlike MRI, OCT allows for measurements of isolated axons as the axons within the RNFL are not myelinated (Fisher, et al., 2006). Therefore, RNFL is commonly used to measure disease progress in multiple sclerosis (Fisher, et al., 2006), retinitis pigmentosa (Walia, et al., 2007), and diabetic retinopathy (Dhasmana, Sah & Gupta, 2016).

As an extension of the Central Nervous System, the retina has numerous similarities to the brain and spinal cord. This is one of the key benefits of using an OCT, as it is a non-invasive means as a proxy to the brain. Due to the transparent nature of the retina, the OCT allows for information at the cellular level of retina. Although the OCT was intended to detect abnormalities and monitor the progression of ocular diseases, increasingly the OCT has been used to study the neurodegeneration of the brain. Mutlu et al. (2017) found that some layers of retinal morphology may be makers of gray and white matter volume in the

brain. RNFL thickness is correlated to cerebral white matter, while the GCL is related to cerebral gray matter. Some literature has explored the use of OCT to measure the relationship between RNFL neurodegeneration and the neurodegeneration of the white matter in patients with Multiple Sclerosis (Scheel, et al., 2014). Gordon-Lipkin and colleagues (2007) found that the RNFL is associated with brain atrophy, but not specifically to gray or white matter of the brain. Cross-sectional studies involving patients with Alzheimer's or cognitive impairment have also found reduced RNFL, additionally the GCL and IPL were significantly reduced in these populations (Cheung et al., 2015; Coppola et al., 2015; Thomson et al., 2015).

2.3. INTELLIGENCE

Intelligence is a critical component of cognitive ability due to its supportive nature of learning and executive function across the lifespan. Executive control (also called cognitive control), is the set of cognitive processes that are a part of the regulation of goal-directed behavior (Botvinick, 2001; Norman & Shallice, 1986). Executive function has three components, shifting between tasks or mental sets, updating and monitoring of working memory representation, and inhibition of prepotent responses (Miyake, et al., 2000). Spearman, (1946) developed the theory of g, modernly known as general intelligence. The theory of g is that when a person does well on one test of intelligence, they should do well on other tests of intelligence. Due to this theory, Spearman was able to create a statistical analysis of general intelligence, where the higher the score, the more intelligence the person has. General intelligence has two subcomponents, crystallized and fluid intelligence. Fluid intelligence can be described as problem solving in novel situations relying on the ability to

react creatively and flexibly to the challenge when prior knowledge of the situation is not beneficial (Horn & Cattell, 1967) and rapidly declines after early adult (Wang & Kaufman, 1993). Alternatively, crystallized fluid intelligence is the ability to gain and utilize knowledge and information throughout the lifespan (Horn & Cattell, 1966). This concept of intelligence is derived from the Cattell-Horn-Carroll (CHC) Theory. The CHC theory includes the work of Raymond Cattell, John Horn, and John Carroll making this theory one of the most comprehensive and empirically supported psychometric theory (Alfonso, Flanagan, & Radwan, 2005). Cattell's original theory of fluid intelligence and crystallized intelligence was developed in the early 1940's and is considered the first precursor of the CHC theory. In the early 1990's Horn built upon Cattell's theory, adding speed, quantitative ability, and a broad reading and writing ability to the theory. Carroll developed his own theory in 1993 called the Three-Stratum Theory which is considered to be the second precursor to the CHC theory. There are four key differences between Cattell-Horn theory and Carroll's theory. The first is Carroll's addition of general ability factor which was not included in Cattell's theory. Secondly, the Cattell-Horn theory separates the concepts of quantitative reasoning and quantitative knowledge. Third, the Cattell-Horn theory included the broad reading and writing ability factor. Finally, Carroll's theory included additional memory factors including short-term, associative, meaningful and free-recall memory abilities (Alfonso, Flanagan, & Radwan, 2005). In the late 1990's, McGrew (1997) attempted to bridge the two models of intelligence into one theory. This combined theory includes 10 broad cognitive abilities, including fluid intelligence, crystallized intelligence, and short-term memory, and 70 narrow abilities. For example, the broad cognitive ability has several narrow abilities including general sequencing reasoning, induction, and speed of

reasoning. Another example of the broad concept of crystallized intelligence includes language development, listening ability, and grammatical sensitivity as some of the narrow abilities.

In Horn's advancements of his theory, he further compared fluid and crystallized intelligence. Crystallized intelligence's subcomponents included the subcomponents of verbal comprehension, mechanical knowledge, experimental evaluation, ideational fluency, and associational fluency. Alternatively, fluid intelligence's subcomponents included inductive reasoning, figural relations, associative memory, intellectual speed, and intellectual level (Horn & Cattell, 1967). These subcomponents were found to be systematically higher for older adults compared to their younger counterparts, due to the nature of educational and acculturation of crystallized intelligence. The temporal cortex appears to play a key role in crystallized intelligence (Colom et al., 2009; Barbey et al., 2012) as well as the dorsolateral prefrontal cortex (Kane & Engle, 2002; Ramnani & Owen, 2004; Wager & Smith, 2003, Colom et al., 2009). However, the scores of fluid intelligence tests were systematically higher for younger adults compared to their older counterparts (Horn & Cattell, 1967). The reasons for fluid intelligence increases until young adulthood are more complex. Horn and Cattell (1967) had several theories, the first being that the neural and physiological structures associated with fluid intelligence reach maturation in young adulthood at which point the adequacy of the physiological structure cannot increase to improve learning at a higher rate. The second theory was that the build-up of small childhood injuries is masked by the rapid neural growth and once these physiological structures reach maturation the long-term limiting influence of these smaller injuries can be seen. Third, older adults are more likely to have large injuries to these structures and these

large injuries are the limiting factor. The final theory is that older adults are not exposed to as much learning as children are at school, however the focus of older adults educating the children which requires them to acquire more collective intelligence of their culture (Horn & Cattell, 1967). Prefrontal cortex (Kane & Engle, 2002) and parietal cortex (Gray, Chabris & Braver, 2003) appear to impact fluid intelligence. The relationship between the prefrontal cortex and the decreased fluid intelligence in older adults may be due to the age-related decline of gray matter volume of the prefrontal cortex (Raz, et al., 1997). Interestingly, Ritchie et al., (2015) found that longitudinal changes in white microstructure were related to the changes in fluid intelligence in older adults.

In sum, there is evidence that obesity impacts both retinal degeneration and intelligence in adults. In addition, there is evidence that different neural tissues are associated with intelligence. However, there is a paucity in the literature regarding the relationship between retinal morphology and intelligence among adults with overweight and obesity.

CHAPTER 3: METHODOLOGY

3.1. PARTICIPANTS

Middle-aged adults (25-45) with overweight or obesity (BMI $\geq$ 25.0 kg/m^2) were recruited from an ongoing dietary intervention. Participants were recruited from the East-Central region of Illinois through the use of flyers posted in public buildings as well as through e-mail listservs. Participants were screened via telephone interview and were excluded if they had a BMI of below 25.0 kg/m^2, had a history of ocular disease (e.g., age-related macular degeneration), neurological diseases (e.g., Attention Deficit Disorder, Attention Deficit Hyperactivity Disorder) , and/or chronic metabolic disease (e.g., Crohn's disease, Ulcerative colitis). If participants were not excluded during the telephone interview, each participant completed an informed consent in accordance with the University of Illinois Institutional Review Board. Following informed consent, participants completed medical and demographic questionnaires. Information from these questionnaires was assessed to ensure participants met the inclusion criteria for the study.

Table 3.1 Inclusion-Exclusion Criteria for Participants

Inclusion	Exclusion
1. 25-45 years of age	Below 25 or above 45 years of age
2. No history of neurological, chronic metabolic, or ocular disease/disorders	Any reported neurological, chronic metabolic, or ocular diseases/disorders
3. Complete body composition scan	Unable to complete DXA scan
4. Complete OCT scan	Unable to complete OCT scan

3.2. PROCEDURES

Participants completed the procedures over two visits to the laboratory. After informed consent and confirming eligibility in the study, trained researchers administered the Kaufman Brief Intelligence Test Second Edition (KBIT-2) and administered OCT assessment in both eyes. During the second visit, participants underwent a whole body Dual-Energy X-ray Absorptiometry (DXA) following a 10-hour fast.

3.3. MEASURES

Retinal Morphometry Assessment

Retinal morphometry assessment was completed with the Heidelberg Engineering Spectralis Optical Coherence Tomography (SD-OCT; Heidelberg Engineering, Heidelberg, Germany). The SD-OCT uses a class one laser to emit infrared light at 870 nm through a superluminescent diode. At a scan acquisition rate of 40 kHz, this noninvasive technology implements an eye-tracking system that adjusts for sudden eye movements. Parameters of

the macular volume and thickness scan include a resolution mode of high speed, 30ºx25º, 768x496 pixels, 61 B-Scans separated by 120 microns. Results from the scan were obtained using central, inner, and outer rings centered around the fovea with respective diameters of 1mm, 2.2mm, and 3.45mm, also known as the clinical macula (**Figure 2, Figure 3)**. The retinal images had each layer segmented with Heidelberg software (version: 6.0.11.0), (**Figure 4)** then trained researchers confirmed segmentation and adjusted segmentation for blood vessels. Due to the high degree of correlation between the left eye and the right eye (*r*'s between 0.71-0.97 all *P*'s<0.01), only right eye data is presented for simplification.

Intelligence Assessment

KBIT-2 is a nationally normed test to assess general intellectual abilities (IQ) for ages 4-90 years old. KBIT-2 is divided into three subtests and takes between 25 and 30 minutes for adults to complete. The first subtest is Verbal Knowledge which consists of 60 questions. The participant sees six images and are asked to choose which image best explains the word, phrase, or question read by the trained researcher. The second subtest is the Matrices, similar to the Verbal Knowledge subtest, participants see six images. The participant is asked to choose which image is most associated with the single stimulus picture or best completes a 2x2, 2x3, or 3x3 matrices. The final subtest is Riddles which consists of 48 riddles read by the researcher. The participant is instructed that each riddle can be answered with a single word answer. For each of the subtests, correct answers are given a score of 1 and incorrect answers are given a score of 0. The total scores of the subtests are converted into standard scores with a maximum of 100. For each subsection, the assessment continues until the participant reaches the end of the subtest or after they have four incorrect answers in a row.

Anthropometrics and Adiposity Assessment

Participants were asked to complete a whole body DXA scan using a Hologic Horizon W bone densitometer (APEX Software version 5.6.0.6; Hologic, Bedford, MA). Height (model 240; SECA, Hamburg, Germany) and weight (WB-300 Plus; Tanita, Tokyo, Japan) were measured before the scan three times and the average of the measurements were used. All participants were asked to fast for 10 hours before their appointment and to remove all metal (i.e., remove jewelry, avoid clothing with metal, etc.) for the scan. All scans were analyzed by one trained researcher.

3.4. STATISTICAL ANALYSIS

Pearson correlation analyses were conducted to determine the contribution of demographic and retinal morphometric measures to the intelligence outcomes. Stepwise hierarchical linear regression models were used to examine the contribution of retinal morphology measures to intelligence measures following adjustment for potential confounding variables. Age, sex, education, and %Fat were entered as step 1 control variables and morphometric measures were added at step 2 in the analyses. The significance of the change in the R^2 value between the two steps was used to judge the improvement in the variance explained once retinal measures were included. The independent contribution of each retinal morphometric measure was assessed by studying the β weight and significance at step 2 when explaining variance in intelligence outcomes beyond that of the demographic variables and adiposity. Data were analyzed using SPSS (SPSS v. 24, Chicago, Illinois) with an *a* threshold of $p = 0.05$.

CHAPTER 4: RESULTS

4.1. PARTICIPANT DEMOGRAPHICS

This sample consisted of 55 participants, ages 25-45 (M=34.33±0.82 years) and was predominantly comprised of females (n=38). Approximately half, (49%) of the sample had an overweight BMI classification (between 25.0-29.9 kg/m^2) and the remaining sample (51%) had an obese BMI classification (≥30.0 kg/m^2). The majority of the sample was comprised of individuals who were in the process of completing or had completed higher education or advanced college degrees (62%).]

Preliminary Person Bivariate correlations (**Table 2**) were conducted to assess any dependent relationships in the sample. Sex (males coded as 1, females coded as 0) was negatively correlated with %Fat (r=-0.73, p≤0.01) and positively correlated with macular volume (r=0.36, p≤*0.01*), RNFL volume (r=0.29, p=0.04), IQ (r=0.34, p=0.10), and fluid intelligence (r=0.39, p≤0.01). Age was positively correlated with center foveal thickness (r= 0.30, p=0.02) and trending for RNFL volume (r=.24, p=0.08). %Fat was negatively correlated with fluid intelligence (r=-0.31, p=0.02) and approached statistical significance for macular volume (r=-0.25, p=0.07) and IQ (r=-0.25, p=0.07).

A summary of the regression analyses for each measure of intelligence (**Table 3**) was conducted to assess the impact relationship of each measure. Step 1 in each of the models adjusted for age, sex, education level, and %Fat and was not statistically significant for IQ (ΔR^2=0.13, p=0.13), crystallized intelligence (ΔR^2=0.06, p=0.55), nor fluid intelligence (ΔR^2=0.16, p=0.06). None of the variables in step 1 were independently predictive of any of the intelligence variables. However, sex approached statistical significance as a predictor of

fluid intelligence (β=0.38, p=0.06) though this relationship was mitigated in step 2 after retinal measures were included (β=0.20, p=0.31).

Including retinal measures at step 2 revealed that RNFL volume was related to IQ (β=0.38, p=0.02), and central foveal thickness (β=0.25, p=0.08) approached statistical significance. GCL was the only independent contributor to the variability in crystallized intelligence (β=0.37, p=0.03) whereas central foveal thickness approached statistical significance (β=0.25, p=0.09). Finally, RNFL volume was the only independent contributor to variance in fluid intelligence (β=0.48, p<0.01).

Table 4.1. Participant characteristics

	All (N=55)
Age, years	34.33 ± 0.82
Sex (F, M)	38,17
Body Mass Index, kg/m^2	32.01 ± 0.78
25.0-29.9 kg/m^2, n (%)	27 (49)
≥30.0, n (%)	28 (51)
Whole body adiposity, %	39.08 ± 1.21
Intelligence Quotient	106.13 ± 1.77
Crystallized Intelligence	102.09 ± 2.10
Fluid Intelligence	108.62 ± 1.63
Education Level	
High School, n (%)	2 (4)
Undergraduate College Degree, n (%)	19 (34)
Advanced College Degree, n (%)	34 (62)
Macular Volume (mm^3)	3.11 ± 0.02
RNFL Volume (mm^3)	0.21 ± 0.00
GCL Volume (mm^3)	0.45 ± 0.01
Center Foveal Thickness (μm)	222.24 ± 2.23

Data presented as mean ± SEM wherever unless indicated otherwise
RNFL, Retinal Nerve Fiber Layer; GCL, Ganglion Cell Layer

Table 4.2. Bivariate correlations between retinal morphology measures and participant characteristics

	Age	Sex	%Fat	Education
Total Macular Volume	0.11	0.36**	-0.25†	0.06
RNFL Volume	0.24†	0.29*	-0.11	0.29†
GCL Volume	0.19	0.22	0.02	-0.02
Center Foveal Thickness	0.30*	0.26†	-0.16	-0.14
Intelligence Quotient	-0.05	0.34*	-0.25†	0.10
Crystallized Intelligence	<0.01	0.23	-0.15	0.08
Fluid Intelligence	-0.03	0.39**	-0.31*	0.11

RNFL, Retinal Nerve Fiber Layer; GCL, Ganglion Cell Layer
†p<0.10
*p<0.05
**p<0.01

Table 4.3. Summary of hierarchical regression analysis for intelligence measures

	Intelligence Quotient			Crystallized Intelligence			Fluid Intelligence		
	β	ΔR^2	Model *p*	β	ΔR^2	Model *p*	β	ΔR^2	Model *p*
Step 1									
Age	-0.05	0.13	0.13	<0.01	0.06	0.55	-0.04	0.16	0.06
Sex	0.38			0.27			0.38		
Education	0.10			0.08			0.10		
%Fat	0.04			0.06			-0.01		
Step 2		0.20	0.01		0.27	0.04		0.18	0.02
TMV	-0.21			-0.12			-0.26		
RNFL	0.38*			0.21			0.48**		
GCL	0.19			0.37*			-0.10		
CFT	0.25†			0.25†			0.16		

†p<0.10
*p<0.05
**p<0.01
TMV, total macular volume; RNFL, retinal nerve fiber layer; GCL, ganglion cell layer; CFT, central foveal thickness; %Fat; whole body adiposity.

CHAPTER 5: DISCUSSION

5.1. DISCUSSION

This study aimed to determine the relationship between retinal morphometric measures and intellectual abilities among adults with overweight and obesity. Consistent with our *a priori* hypothesis, we observed that RNFL and GCL volume were significantly related to higher intellectual ability. Interestingly, these relationships were selective in that RNFL and GCL were related to fluid and crystallized intelligence, respectively. However, we observed that great macular volume and CFT were not significant predictors of any of our measures of intelligence approached statistical significance. Overall, these data indicate that OCT-derived retinal measures are sensitive to intellectual abilities among adults with overweight and obesity.

Obesity has been shown to be related to lower gray matter across several brain regions including prefrontal cortex, temporal, occipital cortex, amygdala, and cerebellum, even after adjusting for obesity-related comorbidities (Kharabian Masouleh, 2016). Given that the retina shares developmental, physiological, and anatomical features with the brain, retinal imaging has emerged as an alternative approach to imaging the neural structures (Ong, 2015; Yau, J.W.Y. et al., 2012; London, 2012). The efficacy for using OCT for neural imaging has gained particular empirical support from studies in neurodegenerative diseases. For example, histopathological and clinical studies have shown that patients with Alzheimer's disease have reduced GCL and RNFL thickness compared to controls (Coppola, et al., 2015). However, recent work has related RNFL and GCL thinning to global and regional cerebral atrophy using MRI among neurologically healthy adults (Ong, 2015; Casaletto, et al., 2017). Ong and colleagues (2015) studied a sample of 60-80-year-olds (N=164) and observed that GCL

thinning was selectively related to reduction in occipital and temporal lobe gray matter volume, while no relationships were observed with white matter. Additionally, in a large population based study (*N*=2,124), Mutlu and colleagues (2017) observed that thinner RNFL and GCL were associated with poorer white-matter microstructure. The RNFL and GCL are thought to be components of the ganglion cell complex with the RNFL comprising axons and the GCL signifying cell bodies. Therefore, it is possible the RNFL may correspond to cerebral white matter while the GCL may reflect cerebral gray matter integrity (Mutlu, 2017). However, to our knowledge, this is the first study to implicate thinner retinal morphometric measures in poorer intellectual ability among adults with overweight and obesity.

Although a considerable body of literature has examined the influence of obesity on brain structure and cognitive function (Smith, 2011; Pannacciculli, 2006) the influence of obesity on measures of intelligence has received comparatively less attention. While a negative influence of obesity on IQ was indicated, this relationship appeared to be mitigated following adjustment of demographic factors (Sorensen, et al., 1982). Therefore, the influence of obesity on intellectual achievement may be driven by multiple health and demographic factors. Studying neuroimaging markers of intellectual abilities is important because intelligence supports higher-order mental processes such as executive function (also known as cognitive control) as well as the acquisition of knowledge and learning across the lifespan (Colom, 2010). Data from the present work indicated increasing adiposity was inversely related to fluid intelligence. However, this relationship was no longer significant once other demographic factors were included in step 1 of the regression models. Importantly, the retinal morphometric measure of RNFL was the primary predictive variable

for fluid intelligence. Previous neuroimaging work has shown that abnormalities in white matter such as hyperintensities influence a variety of cognitive functions, particularly under demyelinating diseases such as multiple sclerosis (Kail, 1998). White matter integrity, as indicated by fractional anisotropy, has also been shown to be related to general intellectual abilities and fluid intelligence (Yu, 2008; Haasz, 2013). If the RNFL is reflective of white matter integrity, the findings of the current study are consistent with these aforementioned studies since we also observed a significant positive relationship between RNFL and fluid intelligence. However, we observed that GCL volume was selectively related to crystallized intelligence. Crystalized intelligence is distinct from fluid intelligence because it refers to the ability to retrieve and use information that has been acquired throughout life (Horn & Cattell, 1968). Unlike fluid intelligence, crystallized intelligence does not exhibit susceptibility to aging and may even improve with age (Craik, 2006; Park, 2009). The implication of the finding from the current study is that GCL reflects intellectual abilities that are acquired through learning across the lifespan. Future studies are needed to determine whether changes in obesity and fat distribution differentially compromise particular intellectual abilities during development and aging.

While GCL and RNFL were found to be predictive of intellectual abilities, we did not observe significant correlations between measures of macular volume and central foveal thickness and intelligence. However, it is worth noting that the association among foveal thickness, IQ, and crystallized intelligence approached statistical significance. It is possible that the influence of foveal thickness on intellectual ability is comparatively smaller, relative to GCL and RNFL, and our sample was not adequately powered to detect the relationships. Nevertheless, the patterns observed (i.e., potential relationships among foveal thickness, IQ,

and crystallized intelligence) would be similar to those observed for GCL. Thus, greater foveal thickness may be protective of intellectual abilities thought to be acquired by learning through the lifespan. Given that previous work has shown that foveal thickness is associated with macular pigment optical density or the accumulation of macular carotenoids (Liew, 2006).

5.2. LIMITATIONS

Although the present study provides novel data linking intellectual abilities to retinal morphometric measures assessed by OCT, there are several limitations worth considering. Longitudinal research studies are necessary to characterize changes in retinal measures and intellectual abilities over extended periods of time. Additionally, our study lacked a comparator group of individuals with a lower weight status. Improving the heterogeneity of the sample by including individuals with varying weight status would provide more comprehensive insights into the relationship between obesity, intellectual abilities, and retinal measures. Finally, we did not account for genetics or other lifestyle factors (e.g., diet and physical activity) that have the potential to contribute to IQ and/or retinal morphology.

5.3. CONCLUSIONS

In conclusion, these findings provide cross-sectional evidence supporting the efficacy or utility of retinal morphometric measures – as measured by OCT – to study IQ among adults with overweight and obesity. Importantly, we were able to demonstrate these relationships in a sample of middle-aged adults since previous work has predominantly focused on older adults and individuals with dementia. Selective relationships were observed between particular retinal measures and different intellectual abilities, known to be differentially affected by aging. These data may set the stage to develop future

hypotheses regarding the interaction between aging and weight status and their influence on gray and white matter and different constructs of intelligence.

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APPENDIX A: INFORMED CONSENT

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Investigating the Effects of Avocado Intake on Metabolism and Cognition: A Systems Approach

Participant Consent Form

Investigators Directing Research:
Naiman Khan, PhD, RD; Hannah Holscher, PhD, RD; Nicholas Burd, PhD, Barbara Fiese, PhD; John Erdman, PhD, Michael De Lisio, PhD, and Margarita Teran Garcia, MD, PhD

You are invited to participate in a research study that will help us understand the relationship between diet, gut function, metabolism, and thinking ability. This form will provide you with information about the study.

If you choose to participate, your involvement in this study will last for about 15 weeks. Today is the first of 8 laboratory visits along with 24 brief meal pick-up visits. Visits will last between 15 minutes (for meal pick up) and 3 to 3.5 hours (for one baseline testing session and one 12-week visit). If you agree to take part in this study, you will eat a pre-prepared meal once a day for 12 weeks. During this time we will measure your eye and metabolic health. We will also ask you to wear a physical activity monitor, complete study questionnaires, perform cognitive tasks, collect six fecal samples, collect 2 urine samples, and record your diet and fluid intake. Before we can collect any data, it's important to confirm that you:

1. Are informed about the procedures and their risks
2. Give your consent voluntarily (i.e., participate because you want to)
3. Know that you can withdraw your consent at any time
4. Are between 25-45 years old
5. Do not have any known food allergies

The procedures and the potential risks and/or benefits are provided below.

What You Will Do in the Experiment:

Baseline Period/Before Study Start:
Baseline Testing Day 1 (Today)
First we will ask you to complete brief health questionnaires and measure your height and weight to assess whether you qualify to be part of this study. We will inform you immediately whether or not you qualify for the study based on the information we collect. If you do not qualify, we will not keep any of your information and you will not receive any payment. We will ask you to answer questions about your medical history so we can evaluate your current health. This will help us determine if you are a good fit for this study and if it is safe for you to participate. We would like to know what medications you take now or have been taking to determine whether you qualify for the study. It is important that

we know about your medications since they can affect your metabolism. We will ask you to complete questionnaires to measure your usual diet intake. Your height and weight will also be measured. You will be asked to complete questionnaires to assess your sleep patterns, attitudes towards eating, and stress and anxiety. You will then be asked to look into a scope for a few minutes and observe and respond to a flickering blue light. We will also ask you to undergo an eye scan. During this scan you will be seated in front of an eye scanner and will be asked to rest your head on a support to keep it still. The scanner will then take an image of the eye without touching it. Scanning typically takes 10-15 minutes. Following this we will measure your visual function by asking you to read letters of different sizes on a chart. These tests will allow us to measure your eye health. After this, we will ask you to complete a task on a computer designed to test your memory. You will be given a 7-day food journal and an activity monitor. You will be asked to record your food intake and wear the physical activity monitor for 7 days. You will be asked to return these items when you return for your next visit. Finally, you will receive containers for stool sample collection. You will be asked to collect one fecal sample and bring it to Freer Hall within 15 minutes after passing. You will also be asked to collect one urine sample on the morning of your next scheduled testing visit. We will provide you with instructions on how to use the collection containers. Overall, we expect today's visit will take about 2.0 hours.

Baseline Testing Day 2

On day 2, you will be asked to come to our laboratory after an overnight fast (at least 10 hours). During the fasting, you will be asked not to eat any foods and or drink anything except water. You will also be asked to refrain from drinking caffeinated beverages during the fast. At the start of your visit, you will be seated in a comfortable chair and your brain activity will be recorded using sensors placed on your scalp and face. A trained staff member will explain where the sensors will be placed before attaching them. The sensors are both painless and harmless, and serve to record electrical signals that are naturally produced by the body. You will then be asked to take part in tasks that involve watching a series of symbols or figures that appear on a computer screen in front of you. You will be asked to press button(s) in response to the symbols or figures. You will then be asked to undergo a glucose tolerance test. For this test you will drink a glucose test beverage and have a catheter inserted into a vein in your arm. The catheter will be inserted with the help of a small needle, which will then be removed. Once the needle is removed, you should feel no sensation from the catheter. A small amount of blood will be collected through the arm catheter before you drink the glucose beverage (0 min) and five times (30, 45, 60, 90, 120 min) afterward. A total of 6 blood samples will be collected for a total amount of ~40 ml of blood. This is around 1/8th of the amount removed during a routine blood donation. After each blood sample is collected, the catheter will be "flushed" with a sterile saline solution in order to prevent blood from clotting in the catheter. A small amount of blood will also be collected (about 4 tablespoons) to measure other health markers such as cholesterol. A small amount of blood will be stored and later analyzed for genetic and biological markers that may be related to diet, body fat, and thinking. Venous blood will be drawn by a trained member of the research team. A topical anesthetic will be applied to your arm before the catheter is inserted. It is expected that your day 2 baseline visit will take 3.5 hours.

Baseline Testing Day 3

On day 3, you will be asked to come to our laboratory after an overnight fast (at least 10 hours). During the fasting, you will be asked not to eat any foods and or drink anything except water. You will also be asked to refrain from drinking caffeinated beverages during the fast. During this visit, we will measure waist circumference and ask you to lie flat on your back on a bone and body composition scanner called DEXA. The DEXA scan uses a small amount of X rays and takes about 5-10 minutes. Due to unknown risks to a fetus, you should not have this test if you may be pregnant. After this, we will measure how much energy your body uses while at rest. For this test you will be asked to lie quietly on a bed in a room for 45 minutes. During this time, there will be a clear canopy over your head and shoulders that will measure the amount of carbon dioxide in your breath. Next, we will look at your liver using an ultrasound machine. This machine is similar to what doctors use to look at babies within the womb. We will place a small amount of gel on your stomach to help us get a clearer picture. This gel is hypoallergenic and washes off very easily. We will then place a small wand-shaped instrument on your stomach. This will allow us to look directly at your liver. Images of your liver will be recorded on a video screen. These tests are for research purposes only and a physician will not be reviewing the results. If any problems with your liver are discovered (a mass, for example) your primary care physician will be notified. This scan will take approximately 10 minutes. Next, we will measure your blood pressure using a blood pressure cuff. Finally, we will

ask you to complete a task on a computer designed to test your memory. Overall, we expect today's visit will take 2 hours.

Intervention Period:
After you have completed baseline testing days, you will be asked to consume a test meal once each day for 12 weeks. The meals will be pre-prepared and contain food items that are typically found in the diet of most Americans (e.g., meats, dairy, legumes, grains, fruits, and vegetables). These meals will be ready for you to eat so you will not need to cook anything. You will be asked to visit Freer Hall to pick up your meals at least twice a week (no more than 10 minutes at a time). You will be asked to record your consumption (yes/no, amount consumed) on the dietary record.

During the 12-week study period, we will ask you to visit our laboratory for testing once a month (i.e., at 4 weeks and 8 weeks). During these visits, we will have you repeat the computer tasks completed on the baseline testing days, a blood draw without the placement of a catheter, the eye test described above, and the DEXA scan. You will also be asked to keep a 7-day food journal and provide one fecal sample during the 4th and 8th week of the study (described above).

Post-Intervention/Conclusion:
At the end of the 12-week period, we will ask you to return to the lab for 3 final visits. The procedures for these 3 visits will be identical to those described for baseline visits 1, 2 and 3.

Risks:
This is a low risk study. All the measurement techniques are commonly used in research studies with adults and the risks are minimal. However, the possible risks are detailed below. There are no known risks of the eye test, but it is possible that your eye may become strained or tired. To minimize this, you can take breaks as needed. During the body scan, you will be exposed to a very small amount of radiation. As part of everyday living, everyone is exposed to naturally occurring radiation that results in a dose of about 200 to 300 millirem (mrem) each year. The effective dose from the DEXA procedure is about one (1) mrem. At this dose, no harmful effects of radiation have been documented, suggesting that the risk is slight. There is no known risk of ultrasound exposure which you incurring during the scan of your liver. NOTE: the ultrasound and DXA scans are not used for diagnostic purposes and we are unable to diagnose disease using the results from your scans; however, you will receive a copy of your scans at the end of the study and we encourage you to share them with your physician.

There may be long-term effects of radiation exposure from DEXA to a fetus. Therefore, if I am pregnant, think I might be pregnant, or am trying to become pregnant I will not be scanned. My initial here indicates that I have no reason to believe I am pregnant at this time AND I am not planning on becoming pregnant over the course of this study.
Initial here _____

There may be some discomfort related to the blood draws and the oral glucose tolerance test, but the blood donation procedure is very common and low risk. There is a one in five chance of bruising where the blood is collected. As with all invasive procedures there is a slight risk of inflammation and infection. There is also an extremely slim chance of sudden death during the blood draws. This risk will be minimized by the use of sterile procedures and equipment at all times. There is also a possibility of dizziness and lightheadedness associated with blood draws. You will be seated or lying down during and after the blood draw to reduce risk of falling. All staff members are trained in First-Aid and certified in CPR. Finally, although it is rare, some people are allergic to avocados. Symptoms of an avocado allergy may include itching, swelling, and swallowing difficulties. In addition to avocados, the meals you will be asked to eat will include other foods that some people are allergic to, including nuts, eggs, dairy, soy, wheat, and fish. If you have any known food allergies, you should not participate in this study.

In the Event of Injury:
In the unlikely event of physical injury resulting from this research study, immediate medical treatment is available from health care providers in the area. However, the University of Illinois does not provide medical or hospitalization insurance coverage for participants in this study, nor will the University of Illinois provide compensation for any injury sustained as a result of participation in this research study, except as required by law. If at any time, day or night, you

APPENDIX A: INFORMED CONSENT (CONT.)

have adverse physical symptoms, you should immediately contact your personal physician or emergency personnel (i.e., dial 911).

Benefits:
There is no medical benefit of taking part in this study. However, participation may contribute to our understanding of how diet, metabolic risk, gut health, and thinking are related. We hope the information learned from this study will benefit the general public by providing information about how the brain and gut are connected.

Confidentiality
Will my study-related information be kept confidential?
Yes, but not always. In general, we will not tell anyone any information about you. When this research is discussed or published, no one will know that you were in the study. However, laws and university rules might require us to tell certain people about you. For example, your records from this research may be seen or copied by the following people or groups:

- Representatives of the university committee and office that reviews and approves research studies, the Institutional Review Board (IRB) and Office for Protection of Research Subjects;
- Other representatives of the state and university responsible for ethical, regulatory, or financial oversight of research;
- Federal government regulatory agencies such as the Office of Human Research Protections in the Department of Health and Human Services
- The financial sponsor of the research, the Haas Avocado Board

Some samples obtained during this study will be stored in the laboratory (maximum 15 years), and may be used for further research. These extra samples are used for analyses that need to be repeated. Also, when publishing, reviewers often ask for additional measures and these samples could be used for this as well. Instead of contacting you later, you are asked to indicate whether you will permit these samples to be used in future research by selecting the appropriate option at the bottom of this form.

Privacy and Rights:

Confidentiality is assured for participant's responses and information. Data will be coded using numbers so that no individual data will be identifiable. If information from this study is published or presented at scientific meetings, your name and other personal information will not be used. Personal information may be given out only if required by law. Organizations that may look at and/or copy your information and responses for research, quality assurance, and data analysis include:

- Government representatives, when required by law
- University of Illinois at Urbana-Champaign Institutional Review Board

Participation in this project is voluntary and you are free to withdraw your participation without penalty at any time.

Cost:
There is no cost to participate in this study. As an incentive for your participation, you will receive a gift card for $50 after completion of testing weeks 4, $100 at week 8, and a gift card for $200 will be provided upon full completion of the study.

Voluntariness:
When you sign this document, you are stating that the study has been fully explained to you, and that you understand that the data from this study are to be used for research purposes only, not for the evaluation or diagnosis of any disorder, and that such data will remain confidential, except as required by law. You are also stating that you have had the opportunity to ask questions about the procedures, that you are aware that participation is voluntary, and that you may withdraw your consent at any time. Your decision to participate, decline, or withdrawal will have no effect on your future relations with the University of Illinois.

APPENDIX A: INFORMED CONSENT (CONT.)

You will be given a copy of this consent form for your records. If at any time (before, during, or after participation) you have a question, you are free to ask it, and you may contact the principal investigators, Dr. Hannah Holscher (217-300-2512, hholsche@illinois.edu) and Dr. Naiman Khan (217-300-1667), nakhan2@illinois.edu), who are responsible for this study. If you wish to speak with someone about complaints or concerns about your *rights as a study participant*, you may contact the University of Illinois Institutional Review Board (217) 333-2670 (E-mail: irb@illinois.edu).

Before you agree to participate, please check each box to indicate that you:

- ☐ Understand the procedure.
- ☐ Give your consent voluntarily.
- ☐ Know that you can withdraw your consent at any time.
- ☐ Have no known food allergies.

I the undersigned, hereby consent to be a participant in the project and undergo testing conducted in the Department of Kinesiology and Community Health and the Department of Food Science and Human Nutrition at the University of Illinois for the following procedures:

- ☐ Venous blood draws
- ☐ Oral glucose tolerance tests
- ☐ Fecal, blood, and urine samples will be stored and coded samples may be provided to other UIUC researchers
- ☐ Additional analyses on the samples collected may be conducted at a later time

I the undersigned, hereby consent to be a participant in the project described above conducted in the Department of Kinesiology and Community Health and the Department of Food Science and Human Nutrition at the University of Illinois.

Participant's Name (Printed): _____

Participant Signature: _____ Date: _____

Signature of experimenter: _____ Date: _____

May we contact you in the future with information about future studies that may be of interest to you?

*Yes*_____ *No*_____

University of Illinois at Urbana-Champaign
Institutional Review Board

Approved: 1-20-17
Expires: 11-17-17
IRB #: 16677

5

APPENDIX B: FIGURES AND TESTING FORMS

Figure B.1: Retinal layers of OCT Scan.

Retinal Layers	
Abbr.	**Name**
ILM	Internal Limiting Membrane
RNFL	Retinal Nerve Fibre Layer
GCL	Ganglion Cell Layer
IPL	Inner Plexiform Layer
INL	Inner Nuclear Layer
OPL	Outer Plexiform Layer
ONL	Outer Nuclear Layer
ELM	External Limiting Membrane
PR	Photoreceptor Layers
RPE	Retinal Pigment Epithelium
BM	Bruch's Membrane
CC	Choriocapillaris
CS	Choroidal Stroma

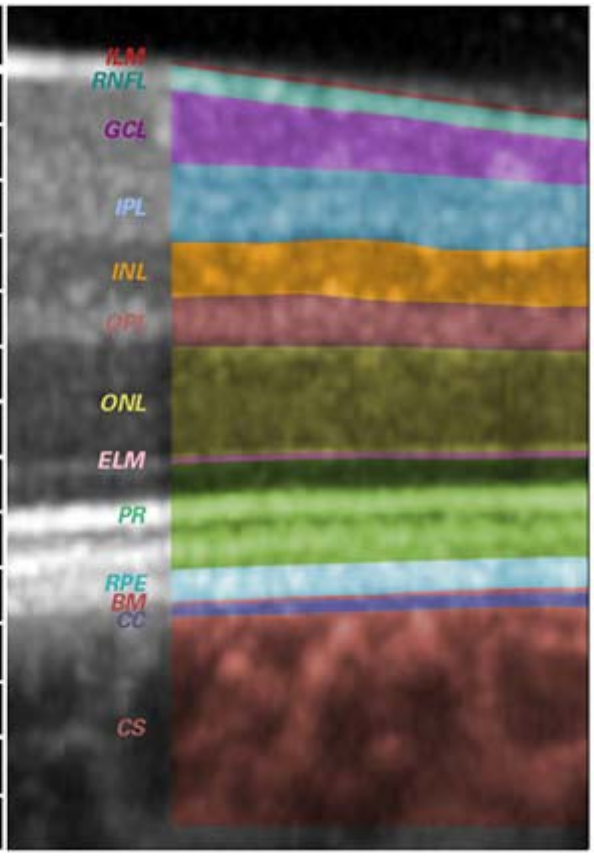

Figure B.2 : Macular scan output Heidelberg Software (version : 6.0.11.0)

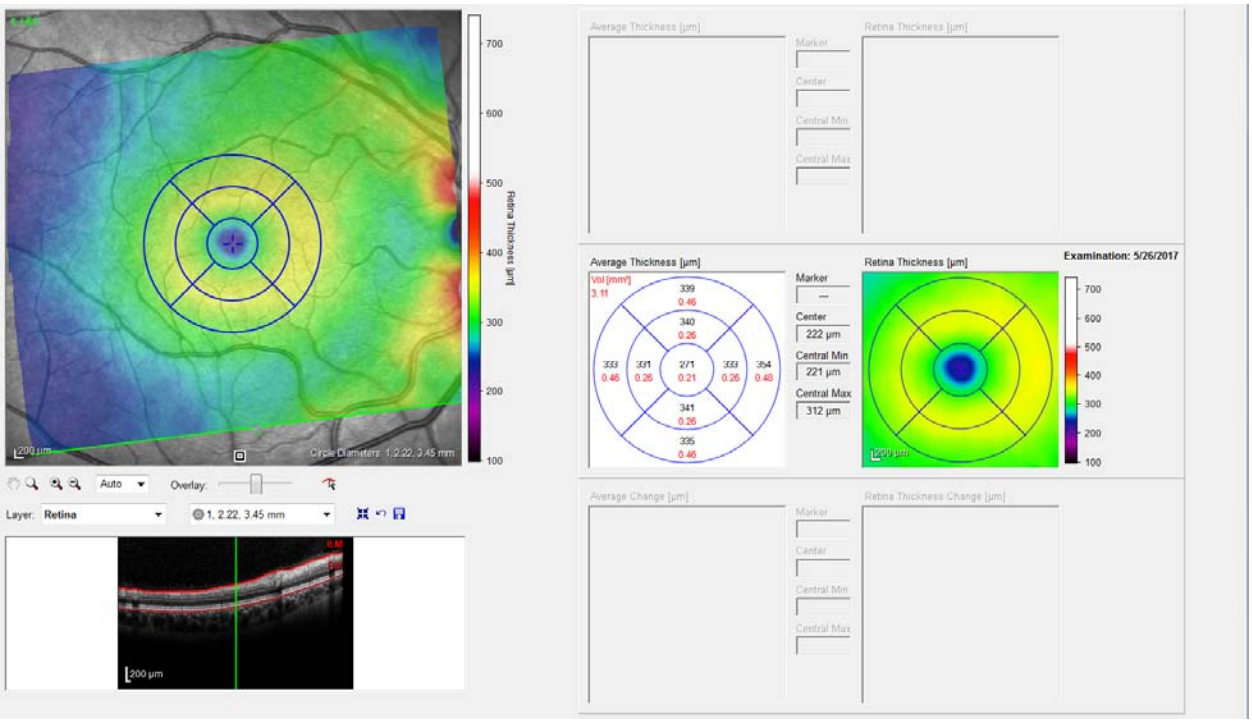

Figure B.3 : Macular scan image

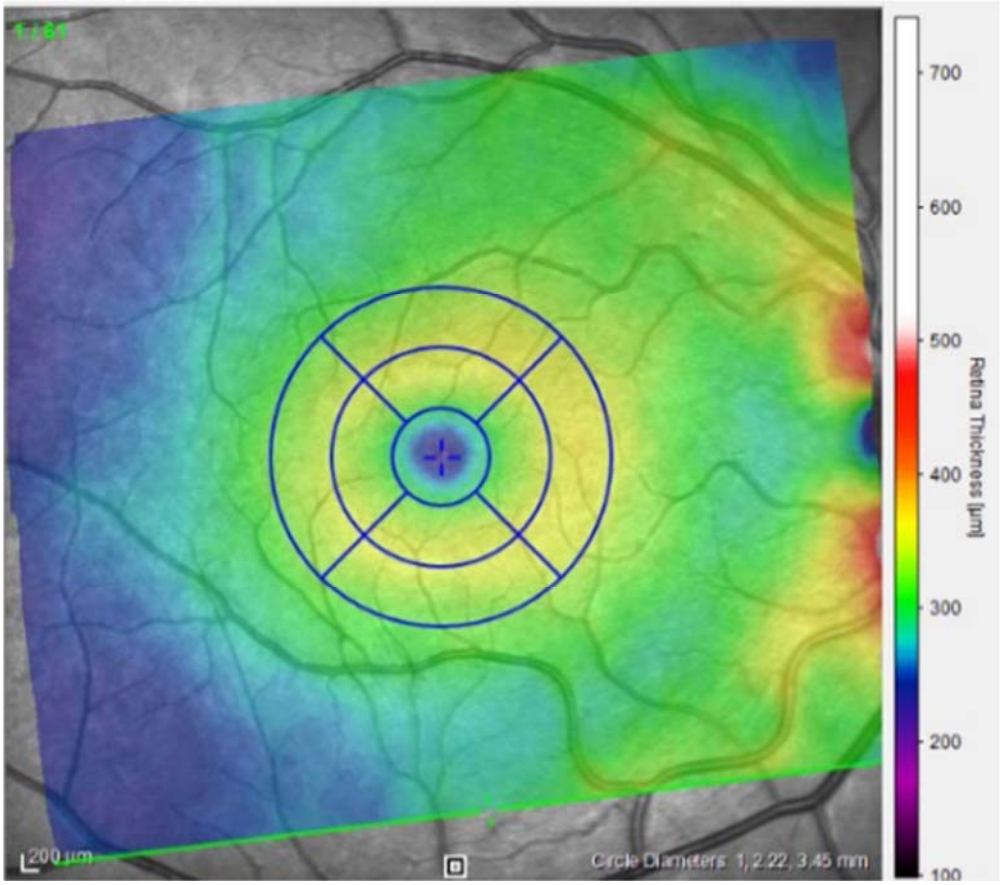

Figure B.4 : Retinal layer separation of RNFL and GCL

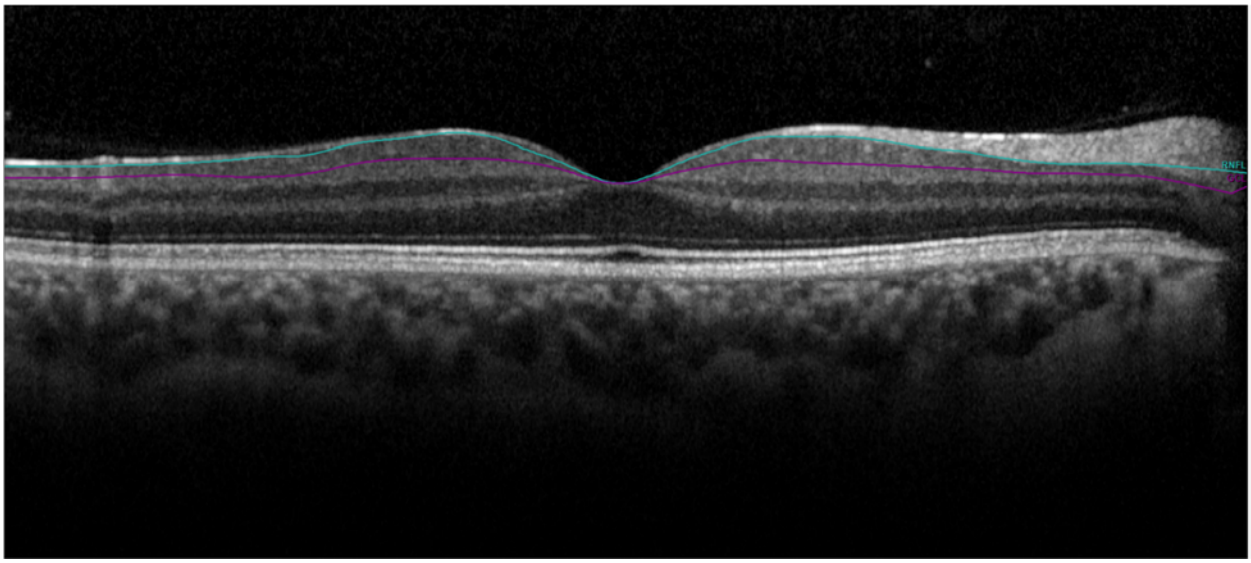

Figure B.5: Kaufman Brief Intelligence test forms

Kaufman Brief Intelligence Test – 2nd Edition Participant:____ Date: _____

Discontinue administration after 4 consecutive incorrect responses

Verbal Knowledge

1. Clock	E	1 / 0	11. What lives in a forest	C	1 / 0	21. What helps you breathe	E	1 / 0
2. Money	E	1 / 0	12. A crowd	A	1 / 0	**22. Athletic**	B	1 / 0
3. Dishes	C	1 / 0	13. Sip	A	1 / 0	23. Railing	C	1 / 0
4. Squeeze	F	1 / 0	14. Reptile	D	1 / 0	24. Canine	D	1 / 0
5. Gift	E	1 / 0	15. Furry	B	1 / 0	25. Elderly	B	1 / 0
6. Storm	B	1 / 0	16. Enter	F	1 / 0	26. Cleanse	C	1 / 0
7. Beaver	D	1 / 0	17. What tells you how much something weighs	A	1 / 0	27. Vocalist	E	1 / 0
8. Shine	D	1 / 0				28. Grasp	F	1 / 0
9. The one that goes with thunder	F	1 / 0	18. Injury	E	1 / 0	29. Victorious	C	1 / 0
			19. Gliding	B	1 / 0	30. Radiant	B	1 / 0
10. Whispering	B	1 / 0	20. Employees	D	1 / 0	31. A famous building in India	E	1 / 0
32. Precipitation	A	1 / 0	42. Discord	B	1 / 0	52. Diminutive	A	1 / 0
33. Informative	A	1 / 0	43. Reproduce	A	1 / 0	53. Mirth	D	1 / 0
34. Recuperate	B	1 / 0	44. The location of the cerebellum	A	1 / 0	54. Seward's folly	F	1 / 0
35. Survey	F	1 / 0				55. Convivial	E	1 / 0
36. Emblem	B	1 / 0	45. Gingerly	C	1 / 0	56. Mollify	D	1 / 0
37. Transaction	D	1 / 0	46. Provisions	B	1 / 0	57. A scene from a john Steinbeck novel	B	1 / 0
38. An important event from the civil rights movement	E	1 / 0	47. Obstruct	C	1 / 0			
			48. Exertion	D	1 / 0	58. Thwarted	B	1 / 0
39. Console	B	1 / 0	49. Gaunt	B	1 / 0	59. A city associated with Carl Sandburg	F	1 / 0
40. Extravagance	B	1 / 0	50. Pliable	A	1 / 0			
41. Illuminate	D	1 / 0	51. What serves a tactile function	D	1 / 0	60. Halcyon	C	1 / 0

Ceiling Item:_____ – Errors: _____ = Raw Score_____

Matrices

~~SampleA.~~ A		~~SampleB.~~ C		19. D	1 / 0	28. E	1 / 0	38. A	1 / 0		
1. C	1 / 0	10. B	1 / 0	20. A	1 / 0	29. F	1 / 0	39. F	1 / 0		
2. B	1 / 0	11. D	1 / 0	21. E	1 / 0	30. F	1 / 0	40. B	1 / 0		
3. D	1 / 0	12. F	1 / 0	22. D	1 / 0	31. E	1 / 0	41. C	1 / 0		
4. E	1 / 0	13. B	1 / 0	~~SampleC.~~ C		32. A	1 / 0	42. F	1 / 0		
5. D	1 / 0	14. E	1 / 0	23. B	1 / 0	33. F	1 / 0	43. C	1 / 0		
6. E	1 / 0	**15. C**	1 / 0	24. F	1 / 0	34. C	1 / 0	44. A	1 / 0		
7. A	1 / 0	16. C	1 / 0	25. E	1 / 0	35. E	1 / 0	45. B	1 / 0		
8. B	1 / 0	17. B	1 / 0	26. A	1 / 0	36. E	1 / 0	46. A	1 / 0		
9. D	1 / 0	18. A	1 / 0	27. C	1 / 0	37. D	1 / 0				

Ceiling Item:_____ – Errors: _____ = Raw Score_____

Riddles – On Back

Ceiling Item:_____ – Errors: _____ = Raw Score_____

Subtests	Raw Score	Standard Score
Verbal Knowledge		
Riddles		
Verbal IQ (Sum of Verbal Knowledge and Riddles)		
Nonverbal IQ		

Riddles

Kaufman & Kaufman (2004) KBIT2

Figure B.5: Kaufman Brief Intelligence test forms (cont.)

1. Socks	1 / 0	4. Boat	1 / 0	7. Foot	1 / 0
2. Peanut	1 / 0	5. Mouse	1 / 0	8. Ice Cream	1 / 0
3. Bed	1 / 0	6. Coin	1 / 0		

9. What is something that wags its tail and barks?	Dog/Puppy	1 / 0
10. What hops, eats carrots, and has long ears?	Rabbit/Bunny	1 / 0
11. What is something round that you put cereal in?	Bowl/Dish	1 / 0
12. What is something shiny and hard that people wear on their finger?	Ring/Nailpolish/Fingernails/ Diamond/Thimble/Splint	1 / 0
13. What is very far away, can only been seen at night, and twinkles in the sky?	Star/Planet	1 / 0
14. What is something that has doors and that you can sleep in at night?	Bedroom/House/Tent	1 / 0
15. What needs daily care, is pink, and holds your teeth in place?	Gums/Braces/Retainer	1 / 0
16. What has a checkout desk, places to read, and rows of books?	Library/Bookshop	1 / 0
17. What is white, is sprinkled on food, and is found in ocean water?	Salt	1 / 0
18. What is fragile, comes in pairs, and is sometimes needed for reading?	Eyeglasses/Lenses/Contacts	1 / 0
19. What is made of rubber, is usually pink, and gets rid of mistakes?	Eraser	1 / 0
20. What melts, burns, and is made of wax?	Candle	1 / 0
21. What has many buttons, has doors, and travels up and down?	Elevator/Airplane/Spaceship/ Helicopter	1 / 0
22. What is made of metal, is different for left-handed people, and is used by barbers?	Scissors/Shears/Clippers	1 / 0
23. What goes around the waist, has no sleeves, and is worn when cooking?	Apron/Smock	1 / 0
24. What has buttons, can be held in your hand, and can solve math problems?	Calculator/Computer	1 / 0
25. What can be seen through, has a sill, and is built into walls?	Window/Doorway	1 / 0
26. What travels long distances, has a whistle, and carries people and goods?	Train/Ship/Boat	1 / 0
27. What is white on top, has a smooth surface, and grows in five places on each hand?	Fingernails	1 / 0
28. What can be walked or driven across, is usually above water, and usually connects two pieces of land?	Bridge/Isthmus	1 / 0
29. What is made of drawings, is seen on television, and sometimes makes people laugh?	Cartoon/Animation	1 / 0
30. What is brown, is made from beans, and is often found in candy bars?	Chocolate/Cocoa/Soy/Vanilla/ Coffee/Carob	1 / 0
31. What is older than books, contains writing, and is rolled up?	Scroll/Parchment/Papyrus/ Scripture	1 / 0
32. What is drawn or written, is seen on walls or buildings, and is unwanted?	Graffiti/Tagging	1 / 0
33. What has a point at the top, sits on sand, and contains mummies?	Pyramid	1 / 0
34. What is sometimes found in lists, makes you pause, and never comes at the end of a sentence?	Comma/Semicolon	1 / 0
35. Who pretends to be someone else, is watched, and has lines?	Actor/Actress/Impersonator	1 / 0
36. What is a type of picture that is painted and shows a person?	Portrait/Caricature/Profile	1 / 0
37. What is made of paper, has one sticky edge, and holds something inside?	Envelope	1 / 0
38. What is nonfiction, is not about the author, and tells the story of a life?	Biography	1 / 0
39. What has the name of a letter, is needed by your body, and is found in food?	Vitamin	1 / 0
40. What hangs around your neck, can be plugged in, and usually has six strings?	Guitar	1 / 0
41. What is seen in a theater, requires a ticket, and happens only during the day?	Matinee	1 / 0
42. What is spoken or written by a sincere person and often follows a blunder?	Apology/Confession/Retraction/ Prayer	1 / 0
43. What is part of a book, has a number, and wouldn't be found in a short story?	Chapter/Footnote	1 / 0
44. What comes from thinking, describes something original, and sometimes solves a problem?	Idea/Solution/Thought/Invention/ Hypothesis/Brainstorm	1 / 0
45. What is sought by collectors, identifies a person, and is sometimes written on unusual objects?	Autograph/Signature	1 / 0
46. What is made of paper, communicates a preference, and is used to determine a winner?	Ballot/Vote	1 / 0
47. What is something that artists perform before a small audience and that results in a selection?	Audition/Tryout	1 / 0
48. What belongs to a person, place, or thing; is informal; and is usually affectionate?	Nickname	1 / 0

Kaufman & Kaufman (2004) KBIT2