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Sex-related differences in oxidative stress and neurodegeneration

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Abstract

Oxidative stress has been implicated in a number of neurodegenerative diseases spanning various fields of research. Reactive oxygen species can be beneficial or harmful, depending on their concentration. High levels of reactive oxygen species can lead to oxidative stress, which is an imbalance between free radicals and antioxidants. Increased oxidative stress can result in cell loss. Interestingly, sex differences have been observed in oxidative stress generation, which may underlie sex differences observed in neurodegenerative disorders. An enhanced knowledge of the role of sex hormones on oxidative stress signaling and cell loss can yield valuable information, leading to sex-based mechanistic approaches to neurodegeneration.

Keywords

Alzheimer’s disease; Parkinson’s disease; testosterone; estrogen; menopause; aging

Oxidative Stress Generation

Oxygen is required by many living organisms for survival. Inefficient oxygen metabolism can be damaging and lead to oxidative stress [1]. Oxidative stress occurs when a biological system is overwhelmed by reactive oxygen species (ROS) due to its inability to counteract these free radicals [2]. Free radicals include peroxides, superoxides, hydroxyl radicals, and singlet oxygen [3, 4]. They are extremely unstable, reacting quickly with many biological products. Under normal homeostatic conditions, ROS plays a role in immunity, homeostasis, and signal transduction pathways [5, 6]. However, when antioxidants are inadequate to balance free radicals and ROS, oxidative stress occurs. These free radicals can trigger lipid peroxidation reactions [7], as well oxidative stress DNA mutations [8]. As the imbalance continues, oxidative stress can induce apoptosis, programmed cell death [9, 10].

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Sex differences in oxidative stress have been observed in numerous basic and clinical studies, wherein males exhibit higher oxidative stress than females [11–17]. Interestingly, sex differences have been observed in the enzyme NADPH oxidase (NOX) [11, 13, 18–20], which is a major oxidative stress generator in cells [21]. NOX is a multi-subunit enzyme, consisting of membrane bound catalytic subunits and cytosolic regulatory subunits. The catalytic subunits are $gp91^{phox}$ and $p22^{phox}$, while the regulatory subunits are $p40^{phox}$, $p47^{phox}$, $p67^{phox}$, and Rac. The regulatory complex translocates to the membrane upon $p47^{phox}$ phosphorylation, leading to subsequent activation of the enzyme [22]. The activated NOX complex catalyzes the transfer of electrons from NADPH to an oxygen molecule, resulting in the formation of reactive intermediates [23]. In different cell types, increased NOX has been observed in males compared to females. For example, using mesenteric arterioles microvessels, Dantas et.al found increased NOX subunits ($p47^{phox}$, $p22^{phox}$, $p67^{phox}$, and $gp91^{phox}$) in young adult male rats compared to females [11]. Similarly, young healthy male rats have increased oxidative stress and NOX expression in aortic and cerebral arterial cells compared to young healthy female rats [19, 20].

Oxidative stress can be influenced by homocysteine. Homocysteine, a non-protein α-amino acid, is an indicator of low folate and B-12 status [24], and has been used as a marker for oxidative stress [25, 26]. Studies have found homocysteine can increase NOX-mediated superoxide production, and this increase in superoxide production can be blocked by the NOX inhibitor, apocynin [18]. Similar to NOX, sex differences in homocysteine levels have been observed. Homocysteine levels are higher in men than women [14, 26–32]. Sex hormones can also influence homocysteine levels. In young healthy transsexuals homocysteine levels were influenced sex hormones. Specifically, male to female transsexuals receiving estrogen hormone therapy experienced decreased homocysteine, whereas female to male transsexuals receiving androgen hormone therapy had increased homocysteine levels [33]. These studies indicate sex hormones may underlie the observed sex differences in oxidative stress generation.

Although males generally have higher oxidative stress than females, oxidative stress is not always damaging. ROS can play a role in homeostasis, such as preconditioning. Preconditioning is a protective process, wherein exposure to a small insult allows the cells to better withstand a subsequent larger insult. This process has been observed in several types of cells (e.g. astrocytes, neurons, fibroblasts, muscle) [34–40]. In our basic science studies, physiological levels of testosterone can increase oxidative stress and be neuroprotective by preconditioning the cell against damage from subsequent exposures to oxidative stress [41, 42]. However, there appears to be a limit in testosterone's preconditioning capabilities. If oxidative stress is too high, then testosterone can be damaging.

Oxidative Stress and Aging

Oxidative stress has been linked with aging [43, 44]. Indeed, one of the major theories of aging is the Free Radical Theory of Aging. This theory proposes oxidative damage to cells, due to a buildup of free radicals in a biological system over a time period, results in aging and aging-associated diseases [45–47]. Sex differences in oxidative stress persist and worsen with aging in both animal and clinical samples, wherein males continue to have higher

oxidative stress than females [48–50]. Aging, specifically menopause, plays a significant role in oxidative stress status in females. Menopause is a period characterized by a dramatic decline of estrogens, which normally occurs around 50–52 years of age in Caucasian women and 48–50 years of age in African-American and Hispanic women [51–57].

As expected, homocysteine increases with age [14, 26–32, 58], indicating elevated oxidative stress [25, 26]. Homocysteine levels further increase with menopause [59–63]. An elegant study by Hak et.al. used aged-matched post- and pre-menopausal women (46–55 years of age) and found homocysteine levels were significantly elevated after menopause [64]. Although other studies have not find this association [65, 66], it could be due a lack of adjusting for age. Similar findings have been observed in animal models, in which downstream targets of homocysteine, such as NOX [18], increased with aging, in general, and in female rats that have undergone ovariectomy, an experimental animal model for menopause [67, 68].

Our laboratory examined the role of aging and homocysteine levels using plasma samples from healthy Caucasian men (n = 700) and women (n = 1,061) over the age of 50 from the Texas Alzheimer's Research Care and Consortium (TARCC) funded by the state of Texas (Table 1). Our data showed homocysteine, used as a marker for oxidative stress, significantly increased with age in both men and women. No differences in homocysteine levels were found between the men and women (Figure 1), which is consistent with prior studies indicating menopause increases homocysteine levels in healthy women to levels observed in healthy men [59–63]. Although hormone manipulations were not examined in the TARCC cohort, estrogen hormone replacement can decrease homocysteine levels in post-menopausal women [69–72]. Interestingly, estrogen hormone replacement therapy in post-menopausal women is associated with decreased testosterone, along with the expected increased estradiol [71, 73, 74]. Generally, menopause is associated with the loss of estrogen, but testosterone levels are maintained [75–78]. The effects of testosterone on menopause are understudied. Since estrogen hormone replacement therapy decreased testosterone levels in post-menopausal women and our prior studies showed that testosterone increased oxidative stress [41, 42], it is possible testosterone may mediate the elevation of homocysteine in post-menopausal women.

In 2001, approximately 40% of post-menopausal women were on some form of hormone replacement therapy [79]. Since there were not any conclusive trial data about the risks and benefits of hormone replacement therapy in post-menopausal women, the National Institutes Health (NIH) sponsored a large randomized, placebo controlled, double blind clinical trial called the Women's Health Initiative (WHI). Participants were post-menopausal women between the age of 50–79 years old at intake. This trial consisted of two arms: estrogen only replacement for post-menopausal women with a prior hysterectomy (n = 10,739) and estrogen + progesterone replacement for post-menopausal women with intact uteri (n = 16,608). Outcomes examined included stroke, venous thromboembolism, cancer, osteoporosis, and coronary heart disease [80]. Results from both arms found no protective effects of either estrogen only or estrogen + progesterone hormone replacement therapy, whereas adverse effects on cardiovascular disease (e.g. pulmonary embolism, deep vein thrombosis, and ischemic stroke) were observed [81]. Due to these negative effects, the WHI

study was terminated early [82]. Based on these results, use of hormone replacement therapy in post-menopausal women drastically declined [83].

Within the past few years, data from the WHI study have been reassessed. Researchers found 83% of the WHI participants were women several years from menopause. Furthermore, the average of age of participants was 63 years old, which is 12–13 years past menopause [84]. When the study outcomes were stratified by age of the participants, the results showed decreased cardiovascular risk and total mortality in younger women with hormone replacement therapy compared to the older women [85, 86]. No evidence of increased stroke was found in women 50–59 years of age in the estrogen only hormone replacement therapy group [87]. Thus, both a women's age and years from menopause are important factors for determining the impact of hormone replacement therapy. These factors are used to determine the "window of opportunity" for hormone replacement therapy in women, wherein the benefit/risk ratio is more protective in women less than 10 years from menopause [88].

Oxidative stress has been proposed as one mechanism underlying the "window of opportunity" for hormone replacement therapy. Several studies found estrogen can decrease oxidative stress [89–91]. However, estrogen's protective effects were found to be conditional [90, 92]. Based on these conditional effects of estrogen, Dr. Roberta Brinton coined the term "Healthy Cell Bias of Estrogen Action." Estrogen exposure to healthy cells results in estrogen being protective against subsequent insults, such as oxidative stress. However, this protective effect is not observed with estrogen exposure in unhealthy cells [90]. Indeed, we have found similar effects with testosterone in our *in vitro* studies [41], indicating the "Healthy Cell Bias" theory applies to all sex hormones in oxidative stress environments. Similarly, clinical studies examining the impact of testosterone replacement therapy found conditional effects of testosterone, in which testosterone replacement therapy was associated with adverse effects in aged men (mean age is 74 years old) with chronic diseases [93, 94].

Oxidative Stress and Neurodegenerative Diseases

Oxidative stress has been implicated in several age-associated neurodegenerative diseases, including Alzheimer's disease [95], Parkinson's disease [96], and non-neurodegenerative diseases (e.g. cancers, sickle cell disease, cardiovascular diseases, and diabetes) [97–100]. Age is one of the greatest risk factors for both Alzheimer's and Parkinson's diseases. Furthermore, oxidative stress is a key feature in these progressive neurodegenerative disorders [96, 101]. Increased oxidative stress has been shown to be involved in cell loss in key brain regions (e.g. substantia nigra, cortex, and hippocampus) involved in the clinical manifestations of Alzheimer's and Parkinson's diseases [96, 102]. Interestingly, sex differences have been observed in both disorders.

Sex Differences in Parkinson's Disease

Parkinson's disease (PD) is a progressive neurodegenerative disorder, which affects millions of people universally. It has been recorded as the second most common neurological disease [103]. One characteristic feature of PD is neuronal death in the substantia nigra of the brain,

specifically dopaminergic neurons. Mechanisms underlying this cell death include oxidative stress and inflammation [41, 104]. This results in the established symptoms of PD relating to the motor system, which include tremor, rigidity, bradykinesia and postural instability [105]. Generally, these symptoms manifest when 80% of the dopaminergic cells within the substantia nigra are lost [106]. The etiology of PD still remains elusive [107]. Aging is one of the principal risk factors for the development of idiopathic Parkinson's disease [108]. Along with aging, sex-related differences in PD have also been recognized [109]. Therefore, it is probable sex hormones play a vital role in this phenomenon, especially as men are 1.5–2 times more at risk of developing PD than women [110–112].

Several studies propose estrogen underlies this sex bias in PD. Women displayed a less severe PD phenotype than men at presentation. Indeed, studies found PD symptoms worsen for pre-menopausal women during menstruation, when estrogen levels are low [113]. Unsurprisingly, severity of PD increases in post-menopausal women compared to pre-menopausal women, due to the loss of estrogen during menopause. Clinical studies found estrogen hormone replacement can diminish the severity of early PD manifestations [114–116]. Therefore, estrogen was proposed to be neuroprotective for dopaminergic neurons in the substantia nigra, and can help mitigate PD progression [117–119].

Contrary to these reports, some studies were unable to find estrogen neuroprotection [120, 121], indicating another mechanism may be mediating this sex difference in PD. One possibility could be testosterone. Currently, the role of testosterone in neurodegeneration is understudied. Few studies have examined the impact of testosterone on PD, compared to studies on estrogen protection. Only one clinical study has been conducted on aged men with PD and treated with L-DOPA, and the results showed testosterone replacement therapy did not impact motor or non-motor PD features [122]. Further, this group observed no interactions between PD medications and testosterone levels [123]. Although this is an understudied area, basic science studies have yielded more information. Increased oxidative stress, via NOX, in the substantia nigral dopaminergic neurons has been reported in male rats compared to female rats [124]. Studies from our lab found testosterone is an oxidative stressor in dopaminergic neurons, and its actions may be involved in this oxidative stress sex difference [41, 42]. In other studies using a 6-OHDA rat model, we observed testosterone can exacerbate oxidative stress damage, resulting in motor impairments [125]. It is possible testosterone may play a role in the increased PD incidence in post-menopausal women compared to pre-menopausal women [112], especially as post-menopausal women are more androgenic than estrogenic [126, 127]. Further research needs to be conducted on testosterone and PD.

Sex Differences in Alzheimer's disease

Oxidative stress plays a key role in the pathogenesis of Alzheimer's disease (AD) [128–130]. Indeed, increased NOX activity has been linked with AD progression and individuals converting from cognitively intact to dementia status [130–132]. Furthermore, associations between homocysteine and AD have been reported. Elevated homocysteine has been shown to contribute to dementia and AD progression [133–141]. Homocysteine can increase oxidative stress and cell loss in the hippocampus (one of the major brain regions affected in

AD) [142]. AD risk is doubled in patients that have greater than 14 umol/L homocysteine, and thus homocysteine has been indicated as a potential AD risk factor [134, 140].

Sex differences have been reported in AD, wherein AD disproportionally affects women more than men in both prevalence and severity [121]. Based on this sex difference, several studies have examined the influence of estrogen on AD. In both *in vivo* and *in vitro* models estrogen protected cells from AD-associated insults [143–149], such as β-amyloid and APP oxidative stress insults [150–152]. Furthermore, estrogen had a positive impact on cognition in surgically menopausal women [153, 154] and post-menopausal women [155–158]. Supporting the role of estrogen in neuroprotection, AD risk increases in post-menopausal women [152]. These studies indicate estrogen can act as a neuroprotectant in AD.

Interestingly, estrogen hormone replacement therapy on cognition in post-menopausal women is equivocal. Subsequent studies based on the WHI included the Women's Health Initiative Memory Study (WHIMS). The effects of hormone replacement therapy on cognition in post-menopausal women were assessed. Unlike the WHI study, the WHIMS study participants were at least 65 years old [92, 159]. Initial results indicated that hormone therapy had a negative impact on cognition. Specifically, estrogen + progesterone was linked with increased dementia and decreased verbal memory [92, 159–161]. Decline in verbal memory is one of the earliest predictors of AD [162, 163]. However, subsequent clinical studies that used peri-menopausal women, instead of post-menopausal women, found estrogen hormone therapy decreased dementia and AD risk [164, 165]. These studies indicate the beneficial effects of estrogen are conditional and may be biased toward protection of healthy cells [90].

Similar to estrogen, studies have indicated androgens can have protective and negative effects on AD. However, it appears testosterone effects are dependent on the cellular environment. One such variable that can result in androgens negatively impacting cells is oxidative stress [41, 166]. Using plasma samples from TARCC participants diagnosed with AD, we found oxidative stress increased with age in both men and women (Figure 2), consistent with other studies [167]. Interestingly, we observed men with AD have higher levels of homocysteine, used as a marker for oxidative stress, than women with AD (Figure 2). In this cohort, hypertension and hyperlipidemia were more prevalent in men with AD than women with AD compared to cognitively intact men and women, respectively. Specifically, 61% (chi-squared $p < 0.05$) of men with AD had hyperlipidemia, unlike women with AD (50%; chi-squared $p = 0.967$). In addition, 58% (chi-squared $p < 0.05$) of men with AD had hypertension, whereas hypertension was present in 51% (chi-squared $p = 0.579$) of women with AD. Both hypertension and hyperlipidemia can increase oxidative stress [11, 168–171], which may increase the oxidative load enough to switch testosterone from a protective hormone to a damaging hormone. Indeed, our prior studies using the TARCC cohort showed endogenous testosterone levels were only associated with cognitive impairment under high oxidative stress (homocysteine levels >12 μmol/L) [172]. This effect of testosterone on cognition was lost when the cohort was not stratified based on oxidative stress, similar to findings in a recent study showing no effects of testosterone replacement therapy on age-associated memory impairment. This study by Resnick et.al. was a large, multi-site, clinical study, in which participants were men over 65 years of age (n = 788) and

exposed to testosterone replacement therapy for one year. Regardless if the men were cognitively intact or impaired prior to hormone replacement therapy, no effects of testosterone were found [173]. No measures of oxidative stress were assayed in this study. Interestingly, majority of the participants in this study were on antihypertensives and phosphodiesterase inhibitors, which decrease oxidative stress [174–180]. Therefore, it is quite plausible testosterone replacement therapy affects cognition in men with elevated levels of oxidative stress.

Conclusion

Sex differences have been observed in oxidative stress and its related diseases. Estrogen and testosterone have been reported to contribute to sex differences in neurodegenerative diseases [181]. Elucidating sex hormone pathways in neurons may provide therapeutic targets to slow down the progression of neurodegenerative disorders by providing sex-based mechanistic approaches. Not only do studies indicate that sex is an important variable in oxidative stress and neurodegeneration, oxidative stress may be a key factor in determining how sex hormones impact neuronal function as either a neuroprotective or neurodamaging agent.

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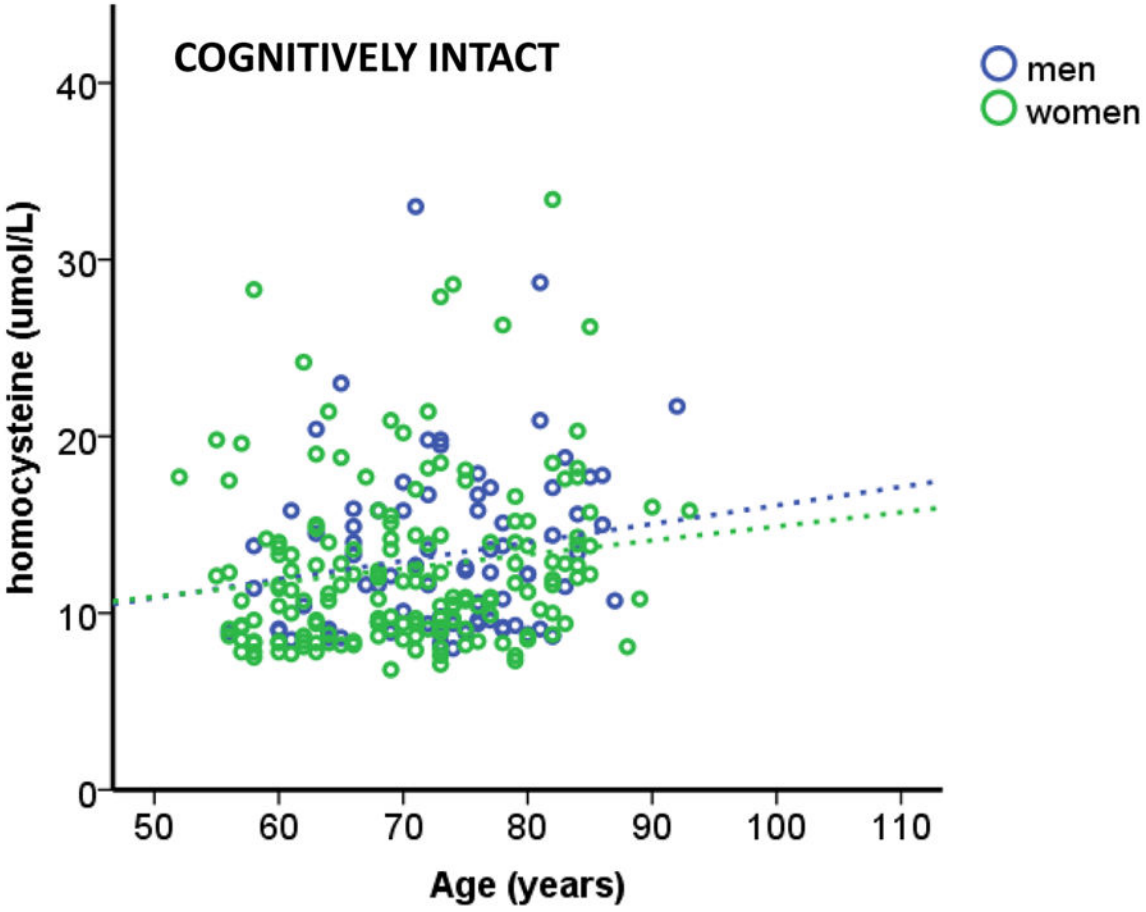

Figure 1.

Homocysteine levels (oxidative stress) increased with age in cognitively intact participants, regardless of sex. A significant regression equation was found ($F(2, 248) = 3.821$, $p < 0.05$), with an R^2 of 0.030. TARCC participants' homocysteine levels are equal to 7.455 + 0.086 (age) − 0.495 (sex), where age is measured in years and sex is coded as men and women. Cognitively intact participants' homocysteine levels increased 0.086 umol/L for every year and men had higher (0.495 umol/L) homocysteine (non-significant) than women. Only age was a significant predictor of homocysteine levels. Specific methods for sample collection are available in our prior publication [26]. Serum total homocysteine was assayed in the Atherosclerosis Clinical Research Laboratory at Baylor College of Medicine.

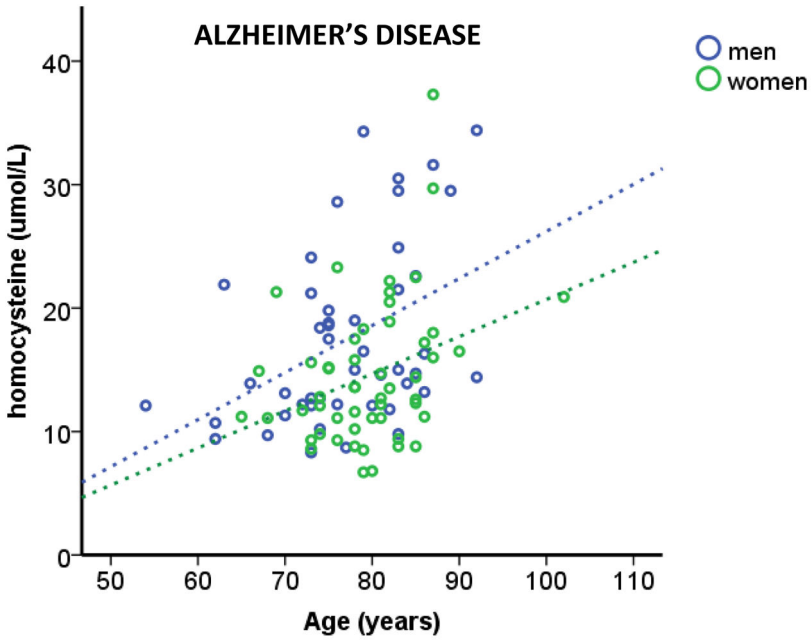

Figure 2.

Homocysteine levels increased with age in both men and women with AD, with men have significantly higher homocysteine levels (oxidative stress) than women. A linear regression was calculated to homocysteine levels based on age and sex. A significant regression equation was found ($F(2, 95) = 11.220$, $p < 0.05$), with an R^2 of 0.191. TARCC participants' homocysteine levels are equal to -5.525 + 0.348 (age) − 3.788 (sex), where age is measured in years and sex is coded as men and women. AD participants' homocysteine levels increased 0.348 umol/L for every year and men had higher (3.788 umol/L) homocysteine than women. Both age and sex were significant predictors of homocysteine levels.

TABLE 1

Sample population characteristics. Plasma samples were obtained from Caucasian men and women enrolled in the Texas Alzheimer's Research Care and Consortium (TARCC). Cognitively intact controls performed within normal limits on all cognitive testing. AD patients met consensus-based diagnosis for probable AD based on NINCDS-ADRDA criteria [182]. Institutional Review Board approval was obtained at each TARCC site and written informed consent was obtained from participants and/or caregivers.

	Men			**Women**		
Variable	**N**	**Mean**	**St. Dev.**	**N**	**Mean**	**St. Dev.**
Age (years)	**700**	**72.71**	**8.93**	**1061**	**72.21**	**9.74**
	min. age		***max. age***	***min. age***		***max. age***
	51.00		**94.00**	**50.00**		**102.00**
	N	**%**		**N**	**%**	
hyperlipidemia	442	*63.14*		578	*54.48*	
hypertension	442	*63.14*		637	*60.04*	
obese	149	*21.29*		264	*24.88*	
Alzheimer's disease	405	*57.86*		516	*48.63*	
cognitively intact	295	*42.14*		545	*51.37*	
< high school diploma	82	*11.71*		168	*15.83*	
high school diploma	98	*14.00*		290	*27.33*	
≤ 4 yrs. college	315	*45.00*		432	*40.72*	
> college	205	*29.29*		171	*16.12*	