Serum Leptin and Age-Related Macular Degeneration

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Purpose. Leptin, a 167-amino acid protein secreted by adipocytes, has been shown to reduce beta-amyloid deposition and intracellular lipid concentration in animal models, two key pathogenic mechanisms underlying aging. We examined the association between serum leptin levels and AMD.

Methods. We conducted a population-based case-control study including Chinese and Indian adults aged 40 to 80 years who participated in the Singapore Epidemiology of Eye Diseases Study (2007–2011). Age-related macular degeneration was assessed from retinal photographs graded using a modified Wisconsin Age-Related Maculopathy Grading System ($n = 426$; early = 389, late = 37). Controls ($n = 927$) without AMD were frequency matched for age, sex, and ethnicity. Serum leptin levels were measured using direct sandwich ELISA.

Results. Participants with AMD had lower levels of leptin compared with those without (mean [SD] = 10.0 [11.5] ng/mL versus 12.9 [16.4] ng/mL; $P = 0.001$). Mean levels of leptin among those with late, early, and without AMD were 8.8, 10.1, and 12.9 ng/mL (P trend = 0.005). In multivariable models adjusting for potential confounders, including smoking, body mass index, blood pressure, and high-density lipoprotein cholesterol, increasing quartiles of leptin were associated with lower odds of AMD, odds ratio (95% confidence interval) of AMD was 0.56 (0.34–0.92) comparing highest to lowest quartile of serum leptin. In subgroup analyses, the inverse association between leptin and AMD was significant in women, Indian ethnicity, and ex-smokers.

Conclusions. Higher serum leptin levels were inversely associated with AMD. These findings, if confirmed in prospective studies, may provide insights into new pathogenic pathways and possibly therapeutic targets in AMD.

Keywords: leptin, AMD, population

Age-related macular degeneration (AMD) is a leading cause of blindness and its prevalence is expected to increase worldwide with aging and increased life expectancy.[1,2] A recent systematic review and meta-analysis estimated 8.7% of the population to be affected by AMD globally, and is projected to increase to approximately 196 million in 2020, and further to 288 million in 2040.[3]

The exact pathogenesis of AMD is unclear. It has even been suggested to be a systemic disease with ocular manifestations.[4] For example, AMD has been suggested to share several clinical and pathological features with Alzheimer's disease (AD), including oxidative stress and inflammation.[5–7] Extracellular deposition of amyloid-β (aβ), the hallmark of AD has also been observed in the drusen of AMD patients. In animal studies, mice deficient in aβ degrading enzyme have been shown to develop subretinal deposits similar to drusen in humans, suggesting that β amyloid deposition could also play a significant role in the pathogenesis of AMD.[8–11]

Leptin, a protein secreted predominantly by the adipose tissue, has been proposed to play a significant role in healthy aging and protection against several neuronal disorders. Acting via hypothalamic receptors, leptin regulates food intake and energy balance.[12–16] Leptin has long been shown to exert adverse vascular changes by accelerating endothelial dysfunction, inflammatory and immune responses, vascular smooth muscle proliferation, and angiogenesis, key pathogenic processes underlying atherosclerosis.[17–19] However, a growing body of evidence suggests beneficial effects of leptin, in particular on lipid metabolism and aging. Leptin has been shown to promote β-amyloid clearance and chronic leptin treatment has been shown to improve memory function in mice models of aging and AD.[20–22] Thus, elevated levels of leptin have consistently been shown to be negatively associated with dementia and AD in several studies,[23–25] including a recent one involving the Framingham study.[26]

It is now known that leptin may be associated with AMD. A small hospital-based study reported that AMD patients had

lower levels of leptin than controls,[27] but this study is limited by a small sample size (32 cases versus 20 controls) and confounder bias. In our current study, we hypothesize that higher levels of leptin may be protective of AMD independent of potential confounders and conducted a population-based case-control study to test this hypothesis.

Methods

Study Population

We designed a case-control study nested within two independent population-based cross-sectional studies, the Singapore Indian Eye Study (SINDI, 2007–2009, $n = 3400$) and the Singapore Chinese Eye Study (SCES, 2009–2011, $n = 3353$). Details of the methodology and population characteristics of SINDI and SCES have been published before.[28] In brief, both studies recruited adults aged 40 to 80 years residing in the southwestern part of Singapore. Both followed similar protocol and were conducted in the same study clinic (Singapore Eye Research Institute). Tenets of the Declaration of Helsinki were followed and ethics approval for both SINDI and SCES were obtained from the Singapore Eye Research Institute Institutional Review Board. Written informed consent was obtained from all participants.

Identification of Cases and Controls

Cases and controls were defined by the presence or absence of any AMD (early or late AMD) determined by grading from fundus photography. For each AMD case, within each 5-year age group, sex and ethnicity strata, approximately two controls were selected from the same study populations. As we had difficulty finding controls for Chinese cases within the age and sex strata, we chose more Indian controls. We identified 426 cases and 927 controls.

Assessment and Definition of AMD

The AMD lesions were assessed from fundus photographs taken using a digital retinal camera (Canon CR-DGi with a 10-diopter SLR backing; Canon, Tokyo, Japan) after pupil dilation. The AMD grading results based on high-resolution digital images, in particular taken after pharmacologically dilating the pupils, have been shown to be comparable to those taken using standard film fundus camera.[29] In brief, two 45-degree retinal images corresponding to Early Treatment for Diabetic Retinopathy Study (ETDRS) standard field 1 (centered on the optic disc) and ETDRS standard field 2 (centered on the fovea) obtained from each eye were graded by the Blue Mountains team at the Centre for Vision Australia, University of Sydney, masked to the participant characteristics following a modification of the Wisconsin Age-Related Maculopathy Grading System (WARMGS). Modifications to the WARMGS protocol were necessary because of a lack of a stereo effect in numerous baseline photographs and different camera magnifications. This system of grading has been described in greater detail elsewhere[30,31] and was chosen to allow comparison with the Blue Mountains Eye Study.[32] The AMD lesions were graded for both eyes, and for each person, the overall severity stage of AMD was assigned based on the eye with the worse grading. For instance, if one eye had late AMD and the other had early AMD, the participant was assigned to have late AMD. Soft drusen were defined as having a diameter larger than 63 μm. Early AMD was defined as either soft drusen alone, RPE abnormalities alone, or a combination of soft drusen with increased retinal pigment and/or depigmentation in the absence of late AMD changes following the definition used in the Blue Mountains Eye Study.[32–34] Late AMD was defined as having either neovascular AMD or geographic atrophy. Neovascular AMD lesions were defined as the presence of RPE detachment; neurosensory detachment; subretinal or sub-RPE hemorrhages; or intraretinal, subretinal, or sub-RPE scar tissue. Subretinal hemorrhages or hard exudates within the macular area also were considered signs of neovascular AMD if other retinal vascular diseases as the alternative causes were ruled out. Geographic atrophy was defined by presence of visible choroidal vessels and a discrete atrophic area with a sharp border with an area of 175 μm in diameter or more. Any AMD was defined as having either early or late AMD. Based on the above grading system, there was no separate category for intermediate AMD.

Measurement of Leptin

Leptin levels were measured from venous blood collected in the nonfasting state at baseline and stored at −80°C. The collection and storage process was the same for cases and controls. Leptin levels were measured by using a commercially available human leptin ELISA kit based on the direct sandwich technique (Millipore, St. Charles, MO, USA) at the National University Hospital laboratory. The sensitivity of the assay was 0.135 ng/mL + 2 SD. Within-day precision was 2.6% to 6.2% for concentration range of 2.34 to 28.00 μg/L and between-day precision was 1.3% to 8.6% for concentration range of 0.86 to 14.08 μg/L.

Assessment of Covariates

Information on participant demographics and personal and medical history were obtained using a standardized questionnaire administered by trained interviewers. Height, weight, and blood pressure (BP) measurements were performed as part of clinic examination. Body mass index (BMI) was calculated as weight in kilograms divided by the height in meters squared (kg/m^2). Obesity was defined as a BMI of 30 kg/m^2 or more. Cigarette smoking was categorized into current, former, and never smoker. Those who had consumed alcohol, irrespective of quantity, were classified as drinkers and those who had never consumed alcohol as nondrinkers. Hypertension was defined as systolic BP of 140 mm Hg or higher or diastolic BP of 90 mm Hg or higher, or self-reported previously diagnosed hypertension. Diabetes mellitus was defined as a random plasma glucose of 200 mg/dL (11.1 mM) or more, or self-reported physician-diagnosed diabetes or use of glucose-lowering medication(s). Cardiovascular disease (CVD) was defined as self-reported myocardial infarction, angina, or stroke. Serum lipids, creatinine, and random glucose levels were measured from venous blood collected in the nonfasting state.

Statistical Analysis

We compared the characteristics of the study participants by case-control status using the χ^2 test or ANOVA as appropriate for the variable. We analyzed leptin levels in quartiles and also as a continuous variable (per SD increase). We examined the association between leptin levels and any AMD adjusting for potential confounders in two logistic regression models. In the first model, we adjusted for age, and sex. In the second multivariable model, we additionally adjusted for history of CVD and traditional CVD risk factors, including ethnicity, BMI, BP, current smoking, ever drinker, total cholesterol, and high-density lipoprotein (HDL) cholesterol that have been shown to be associated with both AMD and leptin in previous literature.[19,35,36] Trends across the quartiles of leptin levels

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Table 1. Characteristics Comparing AMD Cases and Controls

Characteristics	Cases, $n = 426$	Controls, $n = 927$	P*
Age, y	65.8 (10.0)	67.4 (8.9)	0.004
Male, %	249 (58.5)	539 (58.1)	0.9
Chinese, %	234 (54.9)	447 (48.2)	0.02
Diabetes, %	132 (31.0)	320 (34.5)	0.2
Hypertension, %	318 (74.7)	721 (77.8)	0.2
Current smoker, %	46 (10.8)	126 (13.6)	0. 2
Ever drinker, %	51 (12.0)	82 (8.9)	0.07
History of CVD, %	64 (15.0)	184 (19.9)	0.03
Blood glucose, mM	7.16 (3.30)	7.16 (3.42)	0.9
Systolic BP, mm Hg	141.7 (20.2)	141.5 (20.3)	0.9
Diastolic BP, mm Hg	77.4 (9.7)	75.8 (9.4)	0.005
BMI, kg/m^2	24.8 (4.4)	24.9 (4.6)	0.8
Total cholesterol, mM	5.10 (1.09)	5.09 (1.14)	0.9
HDL cholesterol, mM	1.21 (0.39)	1.17 (0.38)	0.08
Leptin levels, ng/mL	10.0 (11.5)	12.9 (16.4)	<0.0001

Data presented are numbers and percentages or means and SDs as appropriate for the variable.

* P value represents difference in characteristics by case-control status based on χ^2 or ANOVA as appropriate.

were examined by using the quartiles as an ordinal variable. To examine the consistency of the association between leptin levels and any AMD, we performed subgroup analysis stratified by potential confounders, including sex, ethnicity, current smoking, and obesity. We examined statistical interaction by stratifying variables by including cross-product interaction terms in the corresponding regression models.

As leptin levels were significantly higher in women compared with men (mean [SD] = 19.7 [18.8] vs. 6.4 [8.1] ng/mL; $P < 0.0001$) consistent with previous literature,[37,38] in a separate analysis, we examined the association of leptin levels with any AMD using sex-specific quartiles of leptin. We then estimated the mean leptin levels by severity categories of AMD using analysis of covariance adjusted for potential confounders. In a supplementary analysis, we tested the association of leptin levels with early AMD, soft drusen, and pigmentary lesion in separate models. All statistical analyses were performed using Stata version 12.1 (Stata Corp., College Station, TX, USA).

Results

Among the 426 participants with AMD, 91.3% ($n = 389$) had early AMD and 8.7% ($n = 37$) had late AMD; among those with late AMD, 92% ($n = 34$) had neovascular AMD. Table 1 shows the characteristics of the study participants. Participants with AMD were more likely to be younger and of Chinese ethnicity, had higher prevalence of self-reported CVD, and had higher levels of diastolic BP than controls. There was no statistically significant difference observed between cases and controls, in terms of the sex, diabetes and hypertension status, smoking or ever drinker, blood glucose level, systolic BP, BMI, total cholesterol, and HDL cholesterol. Importantly, mean serum leptin levels were significantly lower in those with AMD compared with controls (10.0 vs. 12.9 ng/mL, $P < 0.001$).

The association between leptin levels and any AMD is shown in Table 2. Compared with those in quartile 1, those in quartile 4 (highest quartile) of leptin level had significantly lower odds of any AMD in both age, sex-adjusted, and the multivariable models adjusted for potential confounders. This inverse association also was observed when leptin was analyzed as a continuous variable. In subgroup analyses stratified by potential confounders (Table 3), the inverse association between leptin and any AMD was significant in women, people of Indian ethnicity, noncurrent smokers, and those with and without obesity. However, no significant interactions were observed in the association between leptin and AMD by sex, ethnicity, smoking status, or BMI level (all P interaction > 0.05). In Table 4, when we repeated the analysis in Table 2 using sex-specific quartiles of leptin levels, similar to the findings in Table 2, the odds of AMD were significantly lower in quartile 4 compared with quartile 1, with a significant trend across the quartiles in both men and women (P trend = 0.02 in men and P trend = 0.008 in women).

The Figure shows the adjusted mean leptin levels in the whole population by severity of AMD. Compared with those without AMD, mean levels of leptin decreased significantly in a stepladder fashion in early AMD followed by late AMD (P trend = 0.03).

In sensitivity analyses, when we repeated the analyses in Table 2, using early AMD, soft drusen ($n = 405$), and pigmentary lesion ($n = 351$) as outcomes, a similar inverse association was observed between leptin and these outcomes. The multivariable odds ratio (OR) (95% confidence interval [CI]) per SD increase in leptin were 0.69 (0.56–0.86), 0.71 (0.57–0.87), and 0.72 (0.57–0.90) for early AMD, soft drusen, and pigmentary lesion, respectively (data not shown).

Discussion

We report that higher leptin levels were associated with a lower likelihood of AMD in this population-based case-control study. This association was independent of traditional risk factors of AMD, including smoking, BMI, BP, and CVD, and was consistently present when leptin was analyzed either as a categorical or as a continuous variable. In addition, the inverse association between leptin and AMD was consistently present among those with and without obesity. This association persisted for specific features of AMD, including drusen and pigmentary lesions. To our knowledge, this is the first large population-based study examining the association of serum leptin levels with the presence and severity of AMD.

Few previous studies have examined the association between leptin and AMD. In a hospital-based case-control study involving 52 patients, Evereklioglu et al.[27] observed AMD

Table 2. Association of Any AMD With Leptin Levels

Leptin Levels	Cases, $n = 426$	Controls, $n = 927$	Age, Sex-Adjusted OR (95% CI)	Multivariable Adjusted OR (95% CI)*
Quartile 1, 0.8–3.1	122	227	1.00 (reference)	1.00 (reference)
Quartile 2, 3.1–6.7	114	219	0.93 (0.68–1.28)	0.91 (0.66–1.28)
Quartile 3, 6.7–14.5	98	236	0.71 (0.51–1.00)	0.81 (0.48–1.04)
Quartile 4, 14.5–100	92	245	0.60 (0.41–0.87)	0.56 (0.34–0.92)
P for trend			0.004	0.02
Per SD increase, 15.1			0.74 (0.63–0.87)	0.68 (0.55–0.84)

* Adjusted for age, sex, ethnicity, BMI, systolic BP, diabetes, current smoking, ever drinker, total cholesterol, HDL cholesterol, and history of CVD.

Table 3. Association of Any AMD With Leptin Levels (per SD Increase) Within Subgroups

	Cases, $n = 426$	Controls, $n = 927$	Multivariable Adjusted OR (95% CI)*	P Interaction†
Men	249	539	0.72 (0.47–1.10)	0.8
Women	177	388	0.69 (0.54–0.89)	
Chinese	234	447	0.62 (0.38–1.03)	0.6
Indians	192	480	0.68 (0.53–0.86)	
Current smoker: no	380	801	0.67 (0.54–0.83)	0.5
Current smoker: yes	46	126	0.81 (0.42–1.57)	
BMI <30 kg/m^2	375	830	0.75 (0.56–0.99)	0.9
BMI ≥ 30 kg/m^2	51	97	0.51 (0.34–0.75)	

* Adjusted for age, sex, ethnicity, BMI, systolic BP, diabetes, current smoking, ever drinker, total cholesterol, HDL cholesterol, and history of CVD.
† P value associated with the cross-product interaction term between the corresponding stratification variable and leptin in the multivariable model.

patients to have lower levels of leptin than controls. This study was, however, limited by the small sample size and did not account for potential confounders, including BMI and smoking status.

Our findings show that persons with AMD have lower leptin levels, while controlling for multiple potential confounding variables. Importantly, our study is consistent with a growing body of literature that shows a protective association of leptin with AD, cognitive decline, and other neurological disease, such as Parkinson's disease, epilepsy, and ischemic stroke.[26,39–41] In contrast to the above-mentioned neurological diseases, serum leptin has been negatively implicated with multiple sclerosis (MS).[42,43] In one study, the serum leptin levels were significantly higher in MS patients compared with healthy controls, and those with progressive forms of MS were noted to have elevated serum leptin levels compared with those with less severe disease activity. These differences were attributed to the proinflammatory nature of leptin in MS progression. Similarly, serum and cerebrospinal fluid (CSF) leptin levels were shown to be significantly increased in the acute phases of MS, being positively correlated with the increased CSF production of IFN-γ.[44] Leptin's genetic transcript also was found to be elevated in the gene-expression profile of human Th-1 lymphocytes, human Th-1 clones, and active MS lesions in humans. This suggests leptin influences Th-1 responses, which regulate T-cell–mediated autoimmune diseases, such as MS. Leptin also has been shown to dampen regulator CD4+ CD25+ regulatory T cells, thereby promoting autoimmunity.

Age-related macular degeneration has several clinical and pathological characteristics similar to AD. Environmental risk factors, such as smoking, hypertension, hyperlipidemia, and obesity, are shared by both AD and AMD. Both feature plaque formation, inflammation, oxidative stress, and damaged proteasomal and lysosomal function that evoke formation of intra- and extracellular deposits.[5–7] In addition, genetic studies also suggest that both AD and AMD may share common genetic loci in a region of chromosome 7 and associate AMD with AD-associated genes involved in clathrin-mediated endocytosis signaling and atherosclerosis signaling.[45] Thus, our study raises the possibility that leptin may exert a protective effect for AMD, similar to its now more established protective effects against dementia and AD. In addition, it recently has been suggested that leptin could potentially be used as a neuroprotective agent in glaucoma, a neurodegenerative disease characterized by gradual loss of retinal ganglion cells (RGCs), by its ability to prevent RGC death, through reduction of apoptosis, oxidative stress, and excitotoxic damage through numerous molecular pathways.[46]

Several plausible mechanisms have been proposed to explain the inverse association between leptin and AMD. First, leptin has been shown to promote extracellular amyloid clearance[47–49] and β-amyloid deposits characteristic of senile plaques in AD also have been found in drusen deposits.[9,50,51] Second, leptin plays an important role in lipid regulation. As cholesterol accumulation has been shown to contribute to drusen deposits,[52,53] it is likely that increased leptin levels lead to decreased triglyceride and fatty acid synthesis, thereby reducing the amount of intracellular lipid accumulation found within drusen and lesions associated with AMD. Leptin has been shown to specifically inhibit production and enzymatic activity of hepatic stearoyl-CoA desaturase-1, which stimulates the formation of monounsaturated fatty acids.[54] Moreover, the upregulation of leptin genes has been shown to downregulate lipogenic enzyme gene expression, thereby reducing fatty acid synthesis.[55] Third, oxidative stress is an important mechanism implicated in the pathogenesis of AMD.[56] Leptin has been shown to downregulate increased expression of genes related to oxidative stress and inflammation[57] and leptin administration has been shown to reduce oxidative stress in cerebral ischemia in animal studies.[58]

In the current study, we found leptin levels to be significantly higher in women compared with men, a finding consistent with previous literature that also report a two to three times higher concentration of leptin in females compared with males.[37,38] Sex-specific difference in leptin

Table 4. Association of Leptin Levels With Any AMD in Men and Women by Sex-Specific Quartiles of Leptin Levels

Men, $n = 788$		Women, $n = 565$	
Leptin Quartiles	Multivariable Adjusted OR (95% CI)*	Leptin Quartiles	Multivariable Adjusted OR (95% CI)*
Quartile 1, 0.8–2.2	1.00 (reference)	Quartile 1 (0.8–7.1)	1.00 (reference)
Quartile 2, 2.2–4.2	0.55 (0.35–0.86)	Quartile 2 (7.1–13.8)	0.58 (0.34–0.99)
Quartile 3, 4.2–7.6	0.49 (0.30–0.79)	Quartile 3 (13.8–24.7)	0.69 (0.38–1.24)
Quartile 4, 7.6–100	0.52 (0.29–0.90)	Quartile 4 (24.7–100)	0.31 (0.14–0.65)
P for trend	0.02		0.008

* Adjusted for age, ethnicity, BMI, systolic BP, diabetes, current smoking, ever drinker, total cholesterol, HDL cholesterol, and history of CVD.

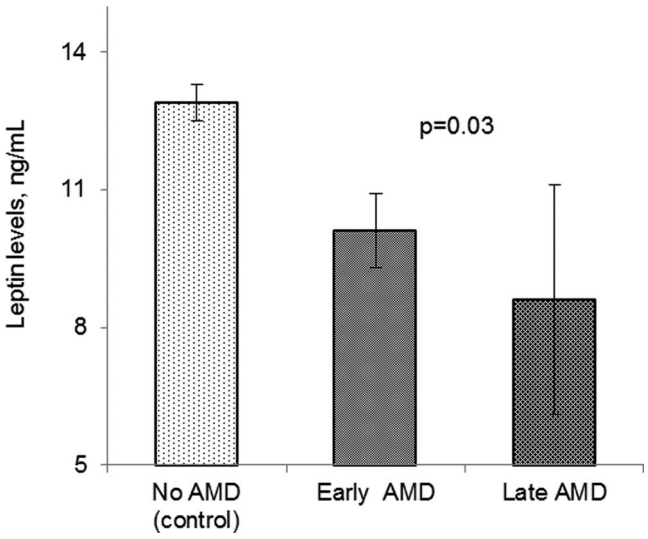

FIGURE. Adjusted* mean leptin levels by severity of AMD. *Adjusted for age, sex, ethnicity, diabetes, BMI, systolic BP, current smoking, ever drinker, total cholesterol, HDL cholesterol, and history of CVD. *Error bars* represent SD. *P* value represents difference in adjusted mean levels of leptin by severity of AMD.

levels could be explained by differences in body fat composition and due to the effect of sex hormones. As the expression of leptin is influenced by adipose tissue, in particular by the subcutaneous adipose tissue, owing to the relatively higher amount of subcutaneous fat in women than in men, women tend to have higher circulating levels of leptin compared with men.[37,38,59] This sex-specific difference in leptin levels associated with adiposity also has been observed in children as young as 5 years, a period of child development without any sex-related hormonal changes. In adults, complex interactions between body fat distribution and reproductive hormones, including testosterone, estrogen, and serum leptin,[60–62] have been postulated to contribute to the sexual dimorphism in leptin levels.

Although past studies show women to have higher incidence of AMD compared with men,[63] more recent studies show the rates of AMD between the two groups are similar and any differences are not statistically significant.[64,65] A current review article also concluded there is no significant association between female sex and late AMD and found inconclusive evidence to support that women have a higher OR compared with men.[2] Consistent with the recent studies,[64,65] in the current study, there was no difference in the percentage of male patients in the AMD and control groups ($P = 0.9$). Our finding of higher leptin levels being inversely proportional to the prevalence of AMD within the same sex group is compatible with current demographic associations.

The strengths of our study include its relatively large sample size, standardized protocols, and information on potential confounders. Our study has some limitations. First, because of the cross-sectional study design, the temporality of the association could not be ascertained. Second, we did not measure vitreous leptin levels to see if they correlated with serum levels and if the association remains unchanged. Third, we defined late AMD based on fundus photographs alone. Fourth, although we had adjusted for several confounders, we cannot exclude the possibility of residual confounding from imprecise measures of smoking and alcohol consumption.

In conclusion, we found persons with AMD had lower serum leptin levels, independent of traditional risk factors. If confirmed by future prospective studies and intervention trials, our findings may have implications for assessing leptin levels in risk-stratification for AMD and leptin supplementation to reduce the burden of AMD.

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