# Precursors of Age-Related Macular Degeneration: Associations With Physical Activity, Obesity, and Serum Lipids in the Inter99 Eye Study

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**Purpose.** To investigate associations of small, hard macular drusen and larger macular drusen with obesity-related risk factors.

**Methods.** Cross-sectional study of 888 subjects aged 30 to 60 years characterized using anthropometric measurements and blood sample analyses. Physical activity was assessed by questionnaire. Digital grayscale fundus photographs were recorded in red-free illumination and graded for the presence of macular drusen > 63 μm in either eye and the presence of 20 or more small, hard macular drusen as a mean of both eyes.

**Results.** Macular drusen > 63 μm were associated with the level of physical activity, the age- and sex-adjusted odds ratio being 0.33 (95% confidence interval 0.13–0.82, $P = 0.016$) for participants who were physically active more than 7 hours/week compared with participants active 0 to 2 hours/week. In women, macular drusen > 63 μm were associated with higher serum triglycerides ($P = 0.0005$). A waist circumference in the top quartile increased the odds for drusen > 63 μm in men whereas in women, having a waist circumference in the middle quartiles reduced these odds. The presence of 20 or more small, hard macular drusen was associated with lower levels of serum high-density lipoprotein cholesterol (HDL; $P = 0.029$) and with moderately elevated triglycerides.

**Conclusions.** Precursors of AMD were associated with modifiable obesity-related risk factors; notably low physical activity with drusen > 63 μm; and lower serum HDL and moderately elevated serum triglycerides with 20 or more small, hard macular drusen per eye. These findings support that a physically active, heart-healthy lifestyle prevents the earliest manifestation of AMD. (ClinicalTrials.gov number, NCT00289237.)

Keywords: AMD, physical activity, drusen, small hard macular drusen

AMD is a multifactorial disease characterized by macular drusen and pigment abnormalites.[1] Genetic predisposition explains approximately 50% of the variation in study population characteristics.[2,3] The dominant genetic factor is the *complement factor H Y402H* polymorphism (*CFHY402H*).[1] Well-documented risk factors for advanced age-related macular degeneration include age[4] and smoking.[5,6] Furthermore, lower physical activity, higher body mass index, larger waist circumference, and increasing waist-hip ratio have been identified as risk factors for AMD.[7–11]

Early AMD is defined by the presence of macular drusen > 125 μm.[4] Drusen smaller than that may be regarded as precursors of AMD. In the Age-Related Eye Disease Study, eyes with at least 1 druse ≥ 63 μm but <125 μm, or more than 15 small drusen (<63 μm) were assigned to AMD category 2, where 316 of 1063 subjects progressed to AMD categories 3 or 4 over a period of 5 years.[12] Small hard drusen are very common in humans[13,14]; and when found in high numbers in the macula, they are associated with an increased risk of developing large soft drusen and fundus pigment abnormalities[15–19] and hence with an increased risk of AMD. Small hard drusen are highly hereditary,[2,20] but so far detailed studies have found no association with AMD genotypes.[21] Associations between small hard drusen and environmental factors remain to be described.

The aim of this study was to investigate associations between obesity-related risk factors and precursors for AMD in the form of each of the phenotypes: multiple small, hard macular drusen (20 of more as a mean of both eyes) and any macular drusen > 63 μm in a cohort of middle-aged subjects.

## Methods

### Study Population

Study participants were recruited from the Inter99 Study. The population-based Inter99 Study is a randomized, nonpharmacological intervention study of ischemic heart disease.[22] The study population consists of inhabitants of a suburban part of Copenhagen from seven birth cohorts (1939–40, 1944–45, 1949–50, 1954–55, 1959–60, 1964–65, 1969–70). In 1999, an age- and sex-stratified random sample of 12,934 eligible

individuals aged 30 to 60 years was invited, of whom 6906 (53.4%) participated. A total of 122 subjects were excluded due to communication problems or alcohol or drug abuse, thus leaving 6784 subjects for analyses. The participants were classified as high or low risk for cardiovascular disease on the basis of a cardiovascular risk score including data on oral glucose tolerance testing; systolic blood pressure manometry; total cholesterol and high-density lipoprotein cholesterol (HDL) assessment; body mass index determination; known diabetes; smoking history; previous cardiovascular disease; and family history of cardiovascular disease.

The Inter99 Eye Study[23] examined a total of 970 subjects from the Inter99 Study in 1999 to 2001. Of that number, 563 represented a population-based sample of all 6784 attendants in the Inter99 Study while 407 subjects represented a sample of all 4053 attendants in the Inter99 Study who were classified as being at high risk of developing cardiovascular disease. The sampling of participants for the eye study was age-stratified into seven age strata corresponding to the seven birth cohorts included in the Inter99 Study. First, a random sample was drawn from the total Inter99 population followed by a random sample from the group of participants classified as high risk for cardiovascular disease. Baseline data were collected from March 1999 to January 2001.

Exclusions made before data analysis comprised 80 individuals whose photographs were missing or of inferior quality in one or both maculae and two subjects with diabetic retinopathy that precluded reliable drusen counting.

All subjects gave their signed informed consent. The protocols were in accordance with the Declaration of Helsinki and approved by the local ethical committees. The Inter99 Study was registered in 2005 at ClinicalTrials.gov (study ID number NCT00289237).

## Ophthalmological Assessments

Best corrected visual acuity in each eye was determined using a Snellen chart after subjective refractioning at a distance of 4 meters. The participants underwent a general ophthalmic examination after pupil dilation with tropicamide 0.5% and phenylephrine 10% eye drops, including fundus photography comprising 7-field monochromatic nonstereoscopic 60°digital fundus photography (TRC-50X camera; Topcon Corp., Tokyo, Japan; with 1024 × 1024 pixel CV-1000 back-piece, Angio-Vision 1000; MediVision, Yokneam Elit, Israel) in red-free illumination (Wratten 54 filter; Eastman Kodak, Inc., Rochester, NY) and fovea-centered stereoscopic color diapositive photography (Ektachrome Elite 100; Eastman Kodak, Inc.).

Fundus characteristics were assessed by a single ophthalmologist (ICM) masked to sex, age, systemic parameters, and genotypes. Digital images were examined on a computer screen and diapositives by using a handheld pair of 15-diopter (D) lenses. Histogram stretching was allowed during the evaluation of the digital photographs. Small hard drusen were defined as any bright element with a diameter equal to or smaller than 63 μm, the shape, color, or proximity to adjacent pathology of which did not suggest that it could be hard exudate, subretinal precipitate, or focal loss of retinal pigment epithelium without drusen formation. This definition excluded drusen associated with nevi. When lesions on digital images were deemed questionable or other retinal pathology was present, color diapositives were inspected and a second ophthalmologist was consulted.

The number of small hard drusen $\leq$ 63 μm was counted within a circle centered on the foveola with a radius stretching to the temporal rim of the optic disc. Within this circle, the presence of drusen $>$ 63 μm was also noted. Both eyes were evaluated. Only subjects with gradable photographs from both eyes were included. The number of small, hard drusen was given as the mean of the subject's two eyes.

## General Assessments

After a minimum of 8 hours of overnight fasting, subjects underwent study procedures including anthropometric measurements, blood samples, and an oral glucose tolerance test. Lipids were determined using enzymatic techniques (Boehringer, Mannheim, Germany). DNA from whole blood was analyzed for *complement factor H (CFH)*, variant *Y402H* (rs1061170). Genotyping was performed by allelic discrimination (TaqMan; KBiosciences, Herts, UK). All genotyping success rates were above 95% (95.2%–98.8%). The distribution of genotypes was in Hardy-Weinberg equilibrium.

Waist circumference was measured midway between the lower rib margin and the iliac crest in the horizontal plane and hip circumference was measured at the point yielding the maximum circumference over the buttocks. Body weight was measured with subjects wearing only light clothes and without shoes. Body mass index was calculated as weight in kg divided by squared height in meters. The lowest value of the two blood pressures measurements with the patient in the supine position was used for data analyses.

Physical activity, measured in hours of activity per week, was assessed by questionnaire. One question was focused on commuting activity and read: "How much time do you spend walking, cycling, or running on your way to and from work?" The answer categories were less than 15 minutes, 15 to 30 minutes, 30 minutes to 1 hour, 1 hour or more, and currently unemployed. Leisure-time physical activity was assessed with the question: ''How many hours a week are you physically active (including walking, bicycle riding, and gardening, but excluding transportation to and from work)?" The response categories were 0 minutes, approximately half an hour per week; approximately 1 h/wk, approximately 2 to 3 h/wk, approximately 4 to 6 h/wk, and 7 h/wk or more. Total physical activity was calculated by summing responses to the questions on commuting physical activity (converted into minutes per week using a 5-day working week) and leisure time physical activity (converted into minutes per week). When the answer categories were intervals, the middle value was used, and when the answer categories were open-ended, the lowest value was used. The answer category "I do not work at the moment" was assigned the value 0 minutes so as not to exclude participants who did not work. The variable total physical activity was grouped into four categories: 0 to 113 min/wk (0–2 h/wk); 143 to 225 min/wk (2–4 h/wk); 255 to 420 min/wk (4–7 h/wk); 450 to 720 min/wk (7–12 h/wk).

Smoking was also assessed by questionnaire; a daily smoker was defined as one with an average consumption of $\geq$7 grams of tobacco per day for more than 1 year.

Categories of lipidemia were defined as described by National Institutes of Health standards.[24]

## Statistical Analysis

Statistical analyses were made using statistical software (SAS version 9.1; SAS Institute, Cary, NC). Means and standard deviations were calculated for continuous variables; median and interquartile ranges were used in case of skewed distributions. In all analyses, variables were treated categorically as presented in tables. Age was grouped according to the ages of the seven birth cohorts from which the participants were recruited: approximately 30, 40, 45, 50, 55, and 60 years. Systolic blood pressure was categorized by the intervals $<$ 130, 130 to 139, 140 to 159, and $>$160 mm Hg, corresponding to clinical guideline thresholds.[25] Multiple logistic regression

**Table 1.** Characteristics of Study Population

| Characteristic | Included, $n = 888$ | Not Included, $n = 5896$ | *P* Value |
|---|---|---|---|
| Age, y | 47.6 (7.7) | 45.8 (7.9) | <0.0001* |
| Male sex, % | 47.4 | 48.9 | 0.42† |
| Danish nationality, % | 97.2 | 94.7 | 0.0018† |
| Daily smokers, % | 36.1 | 35.8 | 0.82† |
| Physical activity, h/wk | | | |
| 7–12 | 11 | 13 | 0.0163† |
| 4–7 | 50 | 52 | |
| 2–4 | 22 | 23 | |
| 0–2 | 16 | 12 | |
| Body mass index, kg/m$^2$ | 27.7 (5.3) | 26.1 (4.5) | <0.0001* |
| Presence of diabetes, % | 22.3 | 3.8 | <0.0001† |
| Waist circumference, cm | | | |
| Males | 96.2 (12) | 92.7 (11) | <0.0001* |
| Females | 84.8 (15) | 79.8 (12) | <0.0001* |
| Waist-hip ratio | | | |
| Males | 0.95 (0.1) | 0.93 (0.1) | <0.0001* |
| Females | 0.81 (0.1) | 0.80 (0.1) | <0.0001* |
| Systolic blood pressure, mm Hg | 133 (18) | 128 (16) | <0.0001* |
| Total cholesterol, mmol/L | 5.8 (1.2) | 5.5 (1.1) | <0.0001* |
| LDL cholesterol, mmol/L | 3.68 (1.0) | 3.47 (0.9) | <0.0001* |
| HDL cholesterol, mmol/L | 1.38 (0.4) | 1.43 (0.4) | 0.0008* |
| Triglycerides, mmol/L | 1.3 (1) | 1.0 (0.7) | <0.0001‡ |
| *CFHY402H,* TT/TC/CC, % | 37/47/16 | 38/47/14 | 0.46† |
| Macular drusen > 63 μm, % | 14.4 | – | – |
| ≥20 small hard macular drusen, % | 13.9 | – | – |

The study population was a subpopulation of participants in the Inter99 Study. Data are presented as mean (SD) or as proportions except for the levels of triglycerides that are presented as mean (interquartile range) due to a highly skewed distribution.

* Student's *t*-test.

† $\chi^2$–test.

‡ Wilcoxon-Mann-Whitney test.

(proc LOGISTIC) was used to estimate associations between risk factors for AMD and the following phenotypes: Macular drusen > 63 μm in either eye and 20 or more small, hard macular drusen (mean of two eyes). Waist circumferences and waist-hip ratios were divided into quartiles separately by sex given the different standards for these parameters in men and women.[26,27] Two-tailed *P* values and 95% confidence intervals were calculated by the use of Wald's test. Trends were estimated as the slope when the categorical variables of interest were treated as quantitative variables and model fit tested by likelihood ratio tests. Tests for interaction between *CFHY402H*, age, sex, body mass index, smoking, recruitment group, and individual risk factors were performed by adding the cross product to the model (*P* for interaction ≤ 0.05). The threshold level of statistical significance was set at $P \leq 0.05$.

## Results

A total of 888 subjects had a complete grading of drusen in both eyes. Twenty or more small, hard macular drusen per eye were present in 13.9% of subjects and macular drusen > 63 μm in either eye were present in 14.4% of subjects (Table 1), of which one in five (21%) also had 20 or more small hard drusen. Hence both characteristics were present at the same time in 3.0% of participants (data not tabulated). Visual acuity in the best eye was 0.8 or better in 98.7% of the study population and only 2 subjects (0.2%) had a visual acuity 0.5 or less (data not tabulated). There was a balanced sex distribution (47.4% males) and 97.2% of included subjects were of Danish nationality (Table 1). In agreement with the prespecified subgroup composition, the eye study participants were slightly older (47.6 vs. 45.8 years); had a higher body mass index (27.7 vs. 26.1 kg/m$^2$); less favorable blood lipid profiles; higher systolic (133 vs. 128 mm Hg) blood pressures; a higher prevalence of diabetes (22.3% vs. 3.8%) and were less active compared with the whole Inter99 Study population (Table 1). The proportion of participants classified as high-risk for cardiovascular disease was the same (29%) in the population-matched recruitment group of the Inter99 Eye Study ($n = 521$) as in the whole Inter99 ($n = 6784$).

A higher level of physical activity was associated with lower odds for the presence of macular drusen > 63 μm (*P* trend = 0.0059; Table 2). Among the most active participants (active 7–12 hours per week) and among the second-most active participants (active 4–7 hours per week), the odds ratio (OR) for having such drusen was 0.33 (95% confidence interval [$CI_{95}$] 0.13–0.82, P = 0.016) and 0.58 (95% 0.34–1.00, P = 0.049), respectively, when comparing with the participants with the most sedentary lifestyle (active 0–2 hours per week), adjusting for age, sex, and recruitment group (Table 2). This association remained significant in the multivariate analysis after also adjusting for total serum cholesterol, triglycerides, waist circumference, smoking, and systolic blood pressure (Table 3). There was no significant interaction between the level of physical activity and recruitment group and the presence of drusen > 63 μm. The association between drusen > 63 μm and level of physical activity was also present among the 521 participants in the population-matched group with OR 0.23 ($CI_{95}$ 0.068–0.75, $P = 0.015$) comparing participants active 7 to 12 hours per week with participants active 0 to 2 hours per week and adjusting for age and sex (Supplementary

**Table 2.** Associations Between Physical Activity, Obesity, and Drusen

| Variable | *n* | Number (%) Subjects With Drusen > 63 µm | OR* (95% CI) | *P* Value | Number (%) Subjects With ≥20 Small Hard Drusen | OR* (95% CI) | *P* Value |
|---|---|---|---|---|---|---|---|
| Physical activity, h/wk | | | | | | | |
| 0–2 sedentary | 133 | 25 (19) | 1 | | 16 (12) | 1 | |
| 2–4 | 184 | 30 (16) | 0.78 (0.43–1.43) | 0.43 | 24 (13) | 1.08 (0.55–2.14) | 0.82 |
| 4–7 | 415 | 53 (13) | 0.58 (0.34–1.00) | 0.049 | 62 (15) | 1.32 (0.73–2.41) | 0.36 |
| 7–12 active | 93 | 7 (7.5) | 0.33 (0.13–0.82) | 0.016 | 8 (8.6) | 0.74 (0.30–1.82) | 0.51 |
| Trend | | | | 0.0059 | | | 0.87 |
| Recruitment group | | | | | | | |
| Population-matched | 521 | 67 (13) | 1 | | 66 (13) | 1 | |
| Cardiovascular disease, high risk | 367 | 61 (17) | 1.17 (0.80–1.73) | 0.42 | 57 (16) | 1.19 (0.80–1.76) | 0.39 |
| Diabetes | | | | | | | |
| No | 667 | 86 (13) | 1 | | 90 (13) | 1 | |
| Yes | 191 | 40 (21) | 1.37 (0.85–2.22) | 0.19 | 28 (15) | 0.91 (0.54–1.52) | 0.72 |
| Daily smoking | | | | | | | |
| No | 562 | 74 (13) | 1 | | 78 (14) | 1 | |
| Yes | 318 | 52 (16) | 1.36 (0.91–2.03) | 0.13 | 43 (14) | 0.95 (0.63–1.43) | 0.80 |
| Systolic blood pressure | | | | | | | |
| <130 mm Hg | 354 | 44 (12) | 1 | | 44 (12) | 1 | |
| 130–139 mm Hg | 208 | 28 (13) | 1.00 (0.59–1.69) | 1.00 | 33 (16) | 1.31 (0.79–2.15) | 0.30 |
| 140–159 mm Hg | 243 | 37 (15) | 0.93 (0.56–1.54) | 0.78 | 31 (13) | 0.91 (0.54–1.53) | 0.72 |
| ≥160 mm Hg | 83 | 19 (23) | 1.33 (0.69–2.56) | 0.39 | 15 (18) | 1.23 (0.62–2.44) | 0.56 |
| Trend | | | | 0.67 | | | 0.90 |
| Body mass index, kg/m$^2$ | | | | | | | |
| <25 | 296 | 37 (13) | 1 | | 38 (13) | 1 | |
| 25–29 | 357 | 47 (13) | 1.00 (0.61–1.63) | 1.00 | 43 (12) | 0.91 (0.56–1.49) | 0.72 |
| ≥30 | 235 | 44 (19) | 1.41 (0.85–2.35) | 0.18 | 42 (18) | 1.37 (0.82–2.27) | 0.23 |
| Trend | | | | 0.17 | | | 0.22 |
| Waist–hip ratio† | | | | | | | |
| First quartile | 226 | 28 (12) | 1 | | 34 (15) | 1 | |
| Second quartile | 225 | 21 (9) | 0.61 (0.33–1.13) | 0.12 | 30 (13) | 0.79 (0.46–1.36) | 0.40 |
| Third quartile | 219 | 36 (16) | 1.12 (0.64–1.95) | 0.70 | 26 (12) | 0.63 (0.36–1.11) | 0.11 |
| Fourth quartile | 216 | 43 (20) | 1.38 (0.79–2.39) | 0.26 | 33 (15) | 0.81 (0.47–1.42) | 0.47 |
| Trend | | | | 0.073 | | | 0.36 |
| | *n* | $P = 0.024$ for interaction with sex | Men, OR* (95% CI) | Women, OR* (95% CI) | $P = 0.87$ for interaction with sex | Men, OR* (95% CI) | Women, OR* (95% CI) |
| Waist circumference | | | | | | | |
| First quartile | 221 | 26 (12) | 1 | 1 | 27 (12) | 1 | 1 |
| Second quartile | 219 | 24 (11) | 1.97 (0.71–5.46) | 0.43 (0.19–0.95) | 32 (15) | 0.93 (0.39–2.19) | 1.24 (0.58–2.65) |
| Third quartile | 232 | 32 (14) | 2.91 (1.13–7.47) | 0.40 (0.18–0.89) | 24 (10) | 0.65 (0.27–1.58) | 0.82 (0.36–1.90) |
| Fourth quartile | 215 | 46 (21) | 3.33 (1.26–8.78) | 1.07 (0.52–2.24) | 40 (19) | 1.02 (0.43–2.41) | 1.75 (0.81–3.77) |
| Trend | | | $P = 0.010$ | N/A‡ | | $P = 0.87$ | $P = 0.25$ |

* Adjusted for age, sex, and recruitment group (and macular drusen > 63 µm).
† Divided into quartiles separately for men and women.
‡ The logit linear assumption was not met.

**Table 3.** Multivariate Analyses

| | Macular Drusen > 63 µm | | ≥20 Small, Hard Macular Drusen | |
|---|---|---|---|---|
| | OR* (95% CI) | *P* Value | OR* (95% CI) | *P* Value |
| Physical activity, h/wk | | | | |
| 0–2 sedentary | 1 | | 1 | |
| 2–4 | 0.66 (0.34–1.25) | 0.20 | 1.09 (0.54–2.20) | 0.81 |
| 4–7 | 0.56 (0.32–0.99) | 0.047 | 1.35 (0.73–2.49) | 0.34 |
| 7–12 active | 0.30 (0.12–0.76) | 0.012 | 0.78 (0.31–1.96) | 0.59 |
| Trend | | 0.015 | | 0.79 |
| Total cholesterol, mmol/L | | | | |
| <5.2 | 1 | | 1 | |
| 5.2–6.2 | 0.69 (0.39–1.24) | 0.21 | 0.71 (0.42–1.19) | 0.19 |
| >6.2 | 1.71 (0.98–3.00) | 0.061 | 0.73 (0.42–1.27) | 0.26 |
| Trend | | N/A† | | 0.27 |
| | Men, OR* (95% CI) | Women, OR* (95% CI) | Men, OR* (95% CI) | Women, OR* (95% CI) |
| Triglycerides, mmol/L | | | | |
| <1.7 | 1 | 1 | 1 | 1 |
| 1.7–2.3 | 0.99 (0.44–2.21) | 1.81 (0.81–4.08) | 3.41 (1.49–7.80) | 2.51 (1.22–5.19) |
| >2.3 | 0.58 (0.24–1.44) | 4.26 (1.59–11.4) | 2.27 (0.91–5.63) | 1.81 (0.63–5.16) |
| Trend | $P = 0.28$ | $P = 0.0036$ | $P = 0.038$ | $P = 0.059$ |
| Waist circumference | | | | |
| First quartile | 1 | 1 | 1 | 1 |
| Second quartile | 2.64 (0.86–8.08) | 0.26 (0.10–0.67) | 0.81 (0.30–2.16) | 1.20 (0.51–2.86) |
| Third quartile | 3.24 (1.11–9.53) | 0.18 (0.06–0.50) | 0.61 (0.22–1.70) | 0.71 (0.26–1.90) |
| Fourth quartile | 3.55 (1.14–11.0) | 0.50 (0.19–1.30) | 1.02 (0.37–2.84) | 1.31 (0.51–3.39) |
| Trend | $P = 0.033$ | N/A† | $P = 0.95$ | $P = 0.77$ |

* Adjusted for age, sex, smoking, systolic blood pressure, recruitment group, and all variables listed (and macular drusen > 63 µm).
† The logit linear assumption was not met.

Table S1). Among the 367 participants in the group with cardiovascular disease high-risk characteristics, the association was not significant, but the tendency was the same with OR 0.58 ($CI_{95}$ 0.14–2.36, $P = 0.45$) comparing participants active 7 to 12 hours per week with participants active 0 to 2 hours per week and adjusting for age and sex (data not tabulated). There was no significant association between recruitment group and drusen > 63 µm (Table 2).

We found a significant interaction between waist circumference and sex (Table 2). In men, the odds of macular drusen > 63 µm increased with increasing waist circumference (*P* trend = 0.010) and the ORs were 3.33 ($CI_{95}$ 1.26–8.78, $P = 0.015$) and 2.91 ($CI_{95}$ 1.13–7.47, $P = 0.027$), comparing participants with a waist circumference in the highest quartile and the second highest quartile, respectively, with participants with a waist circumference in the lower quartile (Table 2). This association was still present in the multivariate analysis (Table 3). For women, however, a waist circumference in the middle quartiles (second or third) reduced the odds for macular drusen > 63 µm with ORs of 0.43 ($CI_{95}$ 0.19–0.95, $P = 0.037$) and 0.40 ($CI_{95}$ 0.18–0.89, $P = 0.026$; Table 2), respectively. This finding was also significant in the multivariate analysis (Table 3). In the analysis including only participants from the population-based recruitment group, men with a waist circumference in the second and fourth quartile had significantly increased odds for drusen > 63 µm, whereas in women, there was no significant associations between waist circumference and the presence of drusen > 63 µm (Supplementary Table S1). The odds for the presence of macular drusen > 63 µm increased with increasing waist-hip ratio without any significant interaction with sex and without reaching statistical significance (*P* trend = 0.073; Table 2).

There was no significant association between macular drusen > 63 µm and daily smoking, diabetes, systolic blood pressure, or body mass index (Table 2). Because 123 participants reported that they had taken antihypertensive medicine within the last week, we repeated the analyses classifying these subjects as having hypertension (systolic blood pressure > 160 mm Hg). This did not change the conclusion (data not tabulated).

Higher levels of serum total cholesterol were associated with increased risk of macular drusen > 63 µm with an OR of 1.65 ($CI_{95}$ 1.01–2.71, $P = 0.046$) when comparing participants with total cholesterol > 6.2 mmol/L with participants with total cholesterol < 5.2 mmol/L, adjusted for age, sex, and recruitment group (Table 4). However, if the 22 participants who reported taking medical cholesterol-lowering agents were classified with hypercholesterolemia (cholesterol > 6.2 mmol/L), the association was no longer significant (OR 1.50 [0.91–2.48], $P = 0.11$; data not tabulated), and it was also not significant in the multivariate analysis (Table 3) or the in analysis including only participants from the population-based recruitment group (Supplementary Table S2). There was no significant association between low-density lipoprotein (LDL) or HDL cholesterol and macular drusen > 63 µm (Table 4).

In women, the odds of having macular drusen > 63 µm increased with increasing levels of triglycerides (*P* trend = 0.0005) with an OR of 3.92 ($CI_{95}$ 1.83–8.38, $P = 0.0004$) when comparing participants with triglycerides > 2.3 mmol/L with participants with triglycerides < 1.7 mmol/L, adjusted for age and recruitment group (Table 4). This association was significant ($P = 0.0036$) also in the multivariate analysis (Table 3) and in the population-based recruitment group (Supplementary Table S2). There was no association in men between

**Table 4.** Associations Between Serum Lipids and Drusen

| Variable | *n* | Subjects With Drusen > 63 μm, *n* (%) | OR* (95% CI) | *P* Value | Subjects With ≥20 Small, Hard Drusen, *n* (%) | OR* (95% CI) | *P* Value |
|---|---|---|---|---|---|---|---|
| Total cholesterol, mmol/L | | | | | | | |
| <5.2 | 259 | 32 (12) | 1 | | 38 (15) | 1 | |
| 5.2–6.2 | 351 | 36 (10) | 0.71 (0.42–1.20) | 0.20 | 45 (13) | 0.80 (0.50–1.29) | 0.36 |
| >6.2 | 277 | 60 (22) | 1.65 (1.01–2.71) | 0.046 | 40 (14) | 0.83 (0.50–1.37) | 0.47 |
| Trend | | | | N/A† | | | 0.48 |
| LDL cholesterol, mmol/L | | | | | | | |
| <2.6 | 123 | 14 (11) | 1 | | 13 (11) | 1 | |
| 2.6–3.3 | 190 | 19 (10) | 0.91 (0.43–1.92) | 0.80 | 38 (20) | 2.16 (1.09–4.29) | 0.028 |
| 3.4–4.1 | 279 | 44 (16) | 1.39 (0.72–2.71) | 0.33 | 38 (14) | 1.21 (0.61–2.39) | 0.58 |
| 4.1–4.9 | 179 | 27 (15) | 1.29 (0.63–2.65) | 0.49 | 17 (10) | 0.79 (0.36–1.73) | 0.56 |
| >4.9 | 89 | 19 (21) | 1.97 (0.90–4.31) | 0.089 | 14 (16) | 1.34 (0.58–3.07) | 0.49 |
| Trend | | | | 0.071 | | | N/A† |
| HDL cholesterol, mmol/L | | | | | | | |
| <1.0 (men); <1.3 (women) | 250 | 33 (13) | 1 | | 44 (18) | 1 | |
| 1.1/1.3 −1.5 | 359 | 53 (15) | 1.20 (0.73–1.97) | 0.48 | 44 (13) | 0.70 (0.43–1.13) | 0.15 |
| >1.5 | 286 | 42 (15) | 0.94 (0.56–1.58) | 0.81 | 35 (12) | 0.57 (0.35–0.95) | 0.030 |
| Trend | | | | 0.79 | | | 0.029 |
| | *n* | *P* = 0.018 for interactions with sex | Men, OR* (95% CI) | Women, OR* (95% CI) | *P* = 0.77 for interactions with sex | Men, OR* (95% CI) | Women, OR* (95% CI) |
| Triglycerides, mmol/L | | | | | | | |
| <1.7 | 591 | 74 (13) | 1 | 1 | 66 (11) | 1 | 1 |
| 1.7–2.3 | 163 | 27 (17) | 1.24 (0.59–2.57) | 1.60 (0.80–3.21) | 35 (21) | 2.12 (1.04–4.35) | 2.44 (1.29–4.61) |
| >2.3 | 133 | 27 (20) | 1.01 (0.48–2.12) | 3.92 (1.83–8.38) | 22 (17) | 1.39 (0.65–2.98) | 2.11 (0.92–4.86) |
| Trend | | | *P* = 0.89 | *P* = 0.0005 | | *P* = 0.25 | *P* = 0.011 |

* Adjusted for age, sex, and recruitment group (and macular drusen > 63 μm).
† The logit linear assumption was not met.

triglycerides and the presence of macular drusen > 63 μm (Table 4).

Analyses of the presence of 20 or more small, hard macular drusen and the level of triglyceride were stratified on sex given the significant interaction between triglycerides and sex for the presence of drusen > 63 μm. Both men and women with a level of triglycerides in the range 1.7 to 2.3 mmol/L displayed significantly increased odds for the presence of 20 or more small, hard macular drusen when compared with participants with triglycerides < 1.7 mmol/L (Tables 3, 4, Supplementary Table S2). However, the odds for having 20 or more small, hard macular drusen were not significantly increased among participants with the highest level of triglycerides (>2.3 mmol/L; Table 4).

Higher levels of HDL were associated with lower odds for having 20 or more small, hard macular drusen (*P* trend = 0.029; Table 4), but this association was not significant in the multivariate analysis (data not presented) or in the analysis including only participants in the population-based recruitment group (Supplementary Table S2). There were no associations between having 20 or more small, hard macular drusen and total cholesterol (Table 4). For LDL, there was an isolated significant association with 20 or more small, hard macular drusen when comparing the second lowest level with the lowest (Table 4). The presence of 20 or more small, hard macular drusen was not associated with physical activity, diabetes, smoking, systolic blood pressure, body mass index, waist-hip ratio, or waist circumference (Table 2).

No statistically significant interactions between the investigated variables and age, body mass index, or smoking were observed (data not tabulated). There was a significant interaction between the recruitment group and *CFHY402H* and the odds for drusen > 63 μm, which has previously been described[21] (*CFHY402H* was significantly associated with drusen > 63 μm only in the recruitment group with high risk for cardiovascular disease). The results of analyses including only the populations-matched recruitment group ($n = 521$) are presented as supplementary material.

## Discussion

This cross-sectional study of 888 middle-aged subjects found that modifiable obesity-related risk factors in the form of lower physical activity, higher waist circumference in men, and higher level of triglycerides in women were associated with the presence macular drusen > 63 μm, an immediate precursor of AMD. In women, a waist circumference in the middle quartiles was protective of drusen > 63 μm. By convention, AMD is defined by the presence of drusen > 125 μm or more advanced lesions in eyes where drusen have once been present.[4] Having 20 or more small, hard macular drusen per eye, another precursor of AMD,[17] was associated with lower levels of HDL and with moderately increased triglycerides.

There was a strong association between macular drusen > 63 μm and physical activity. We were able to show a dose-

response effect where more physical activity significantly reduced the odds for having precursors of AMD and we found no interaction with *CFHY402H*. The association was also present when only the population-based group of participant was included in the analyses and thus was not a result of the skewed ascertainment for the study, although a general selection bias for the Inter99 Eye Study cannot be accounted for. Nearly all participants had a visual acuity in the best eye of 0.5 or better and hence the association was not a result of subjects with lower visual acuity being more passive. The finding indicates that a physically active lifestyle reduces the risk of developing the earliest stages of AMD also in genetically predisposed people. Lower physical activity has previously been identified as a risk factor for early AMD in some[7] but not all studies.[28–30] Several prospective studies have identified a protective effect of vigorous exercise in relation to incident self-reported AMD,[11] progression of AMD,[8,10] development of geographic atrophy,[10] and development of neovascular AMD.[10,31]

The present study tested the associations between macular drusen $>$ 63 μm and measures of obesity and found that a larger waist circumference increased the odds for having drusen $>$ 63 μm in men only, whereas a waist circumference in the middle quartiles reduced the odds for drusen $>$ 63 μm in women. These findings are not consistent with prior reports where a higher waist-hip ratio was found to increase the odds for early AMD in women only[32] or where a higher waist circumference and also a higher waist-hip ratio was associated with an increased risk of progression of AMD.[8] Furthermore, a reduction in waist-hip ratio over 6 years has been found to be associated with a reduced risk of AMD.[9] The reasons for the discrepancies may relate to differences in study populations such as age, nationality, and differences in outcome (precursors of AMD [present study], early AMD,[32] or progression of AMD).[8] However, we did observe a tendency for increasing odds for drusen $>$ 63 μm with increasing waist-hip ratio with no significant dependency on sex. The lack of statistical significance of this association may be due to the limited power of the study. Body mass index was not associated with drusen $>$ 63 μm in our study, but has previously been found to be associated with late AMD[28,33] and with early AMD,[29,32] however, not in all studies.[30]

Total cholesterol above 6.2 mmol/L was associated with the presence of macular drusen $>$ 63 μm, but the association was not significant when accounting for medical treatment for hypercholesterolemia. This finding is in line with many previous studies that have not found any association between early AMD and the levels of total cholesterol or LDL.[10,30,34–36] Serum cholesterol has been inconsistently associated with late AMD.[37]

The association between serum triglycerides and the presence of drusen $>$ 63 μm was dependent on sex, with serum triglycerides $>$ 2.3 mmol being associated with increased odds for drusen $>$ 63 μm in women only. The effect of sex on the association may reflect an increased susceptibility in women to damage associated with high serum triglyceride with respect to drusen formation, or may be a chance finding resulting from multiple testing. Serum triglycerides have been found to be associated with neovascular AMD,[28] but other studies have not found serum triglycerides to be associated with AMD in general.[33–35,38]

The prevalence of having 20 or more small, hard macular drusen was associated with moderately elevated serum triglycerides in both men and women, but not with the highest levels of serum triglycerides. As the association did not display a dose-response effect, it appears less likely to be biologically significant and should be tested in other studies. The difference in odds for drusen between the two lower categories of LDH is likely to be a chance finding since no other levels of LDH differed significantly.

We found higher serum HDL to be associated with a lower prevalence of 20 or more small, hard macular drusen, although not in the multivariate analysis. Higher serum HDL has been associated with increased prevalence[33] and incidence of AMD,[30,36] but also with lower odds of early AMD[30] and with lower risks of late AMD.[30,35,38] Discrepancies between studies may relate to differences in definitions of AMD, endpoints, and age distribution of cohorts.[30] A single nucleotide polymorphism in the gene *LIPC* coding for hepatic lipase has been linked with advanced AMD,[39,40] but the level of HDL does not seem to mediate the effect of this polymorphism.[38]

Overall, the associations of the presence of drusen $>$ 63 μm and 20 or more small, hard macular drusen differed, except with respect to the level of triglycerides. Notably, there was no association between physical activity and having 20 or more small, hard macular drusen, whereas the association was prominent for drusen $>$ 63 μm. The presence of 20 or more small, hard macular drusen has a much higher heritability than larger drusen[2] and small, hard drusen are not associated with *CFHY402H*.[21] The pathogenesis of small, hard macular drusen may differ markedly from that of drusen $>$ 63 μm.

Diabetes was not associated with precursors of AMD, which is in agreement with previous studies.[33,41]

This study included assessment of hours of current physical activity status by a questionnaire. The questionnaire has been shown to be associated with biological risk factors for cardiovascular disease and with incident diabetes in the Inter99 Study,[42,43] but it has not been validated against objective measures of physical activity. Information about past physical activity would have been relevant, but it was not assessed in the Inter99 Study due to the expected recall and social desirability bias. A strength of the study was the grading of drusen on red-free, digital grayscale fundus photographs, which provide better contrast than color fundus photographs. While our use of 60° fundus photographs rather than the standard 30° photographs[44] may have limited our ability to detect small lesions, we nevertheless found a prevalence of early age-related maculopathy that was indistinguishable from previous studies.[2] A major concern with the study was the planned overrepresentation of subjects with a high risk of cardiovascular disease—which was accounted for, however, by including the recruitment group in all analyses and testing for interaction between variables and the recruitment group. Furthermore, we also performed the analyses separately in the population-based recruitment group to enable assessment of the effect of the skewed ascertainment for the study. The power to exclude associations was limited by the total number of 128 cases of macular drusen $>$ 63 μm and 123 cases of 20 or more small, hard drusen and hence associations may have escaped detection. Furthermore, the number of subjects with drusen was too small to justify classification and analysis by subtype (intermediate, confluent, reticular, etc.). Finally, no conclusion about causality can be made because of the cross-sectional design of this study.

In conclusion, precursors for AMD were associated with modifiable obesity-related risk factors, notably associations of lower physical activity with macular drusen $>$ 63 μm and lower serum HDL, and moderately elevated serum triglycerides with 20 or more small, hard macular drusen per eye—a trait that has previously been found to be determined overwhelmingly by genetic factors.[2,20] These findings support a physically active, heart-healthy lifestyle as a preventive measure for the earliest signs of AMD.

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