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Decline in LDL-cholesterol concentration: lipid lowering drugs, diet, or physical activity? Evidence from the Whitehall II study

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ABSTRACT

Objective: To examine the association of lipid lowering drugs, change in diet and physical activity with decline in LDL-cholesterol in middle age.

Design: Prospective cohort study.

Setting: Whitehall II study.

Participants: 4,469 British civil servants (72% men) aged 39 to 62 years at baseline.

Main outcome measure: Change in LDL-cholesterol concentrations between the baseline (1991-1993) and follow-up (2003-2004).

Results: Mean LDL-cholesterol decreased from 4.38 to 3.52 mmol/L over a mean follow-up of 11.3 years. In a mutually adjusted model, decline in LDL-cholesterol was greater among those who were taking lipid lowering treatment at baseline (-1.14 mmol/L, n=34), or started treatment during the follow-up (-1.77 mmol/L, n=481) compared to untreated individuals (n=3954) ($p<0.001$); among those who improved their diet – especially the ratio of white to red meat consumption and the ratio of polyunsaturated to saturated fatty acids intake – (-0.07 mmol/L, n=717) compared to those with no change in diet (n=3071) (p=0.03); and among those who increased physical activity (-0.10 mmol/L, n=601) compared to those with no change in physical activity (n=3312) (p=0.005). Based on these estimates, successful implementation of lipid-lowering drug treatment for high-risk participants (n=858) and favourable changes in diet (n=3457) and physical activity (n=2190) among those with non-optimal lifestyles would reduce LDL-cholesterol by 0.90 to 1.07 mmol/L in the total cohort.

Conclusions: Both lipid-lowering pharmacotherapy and favourable changes in lifestyle independently reduced LDL-cholesterol levels in a cohort of middle-aged men and women, supporting the use of multifaceted intervention strategies for prevention.

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INTRODUCTION

Blood cholesterol, low density lipoprotein (LDL) cholesterol in particular, is a major risk factor for coronary heart disease (CHD) (1). Large randomized controlled trials and meta-analyses (2-4) have established the clinical benefits of lowering LDL-cholesterol. A decrease of one millimole per litre (mmol/L) in LDL-cholesterol concentrations has been shown to be associated with 23% lower risk of myocardial infarction or coronary death (4). Similarly, a 1% reduction in total cholesterol was associated with 2% reduction in risk of CHD (5).

There is now consistent evidence for a secular decline in total cholesterol and LDL-cholesterol levels among adults in industrialized countries (6-19). For example, the MONICA study showed total cholesterol in adults aged 35 to 64 years to have declined between the mid-1980s and mid-1990s in approximately half of the European populations included in the study (6). Similar findings have been reported in other European populations (8-12), the United States (13-16), Canada (17), Australia (18), and New Zealand (19).

Clinical guidelines recommend a multifaceted approach to lowering LDL-cholesterol (1, 20). Besides medication, this consists of reducing the intake of saturated fats and cholesterol, reducing weight, and increasing physical activity in order to reach optimal levels of LDL-cholesterol. However, the extent to which a healthy diet, physical activity and lipid lowering drugs independently explain the decline in LDL-cholesterol levels currently being observed at the population level is unknown. We therefore examined associations of lipid-lowering drug use and 11-year change in diet and physical activity with declining LDL-cholesterol trends in an occupational cohort of middle-aged British civil servants participating in the Whitehall II study.

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SUBJECTS AND METHODS

Study sample

The Whitehall II study is a prospective cohort study of 10,308 (67% men) London-based British civil servants aged 35-55 years in 1985 (21). The baseline examination (phase 1) took place during 1985-1988 and involved a clinical examination and self-administered questionnaire. Subsequent phases of data collection have alternated between postal questionnaire alone (phases 2 (1988-1990), 4 (1995-1996), 6 (2001), and 8 (2006)), and postal questionnaire accompanied by a medical screening (phases 3 (1991-1993), 5 (1997-1999), and 7 (2002-2004)).

Detailed lipid data were not available at phase 1 so the data used in the current analysis were drawn from phases 3 to 7, making phase 3 the baseline for the present study. Mean follow-up between phases 3 and 7 was 11.3 years (standard deviation, SD=0.5). Participants not included in the analysis were those who did not undertake the medical screening at phases 3 or 7, and those with missing data on any of the predictors (lipid lowering drugs, diet, and physical activity) or potential confounders (ethnicity, body mass index, level of education, smoking status, and presence of longstanding illness) either at phase 3 or phase 7 (**Figure 1**). A total of 4,469 participants were eligible and constituted the study sample. Ethical approval for the Whitehall II study was obtained from the University College London Medical School Committee on the ethics of human research (London, UK).

Biochemical analyses

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Blood samples were collected at phases 3 and 7, following either an 8-hour overnight fast (participants presenting to the clinic in the morning) or at least a 4-hour fast after a light fat-free breakfast (participants presenting in the afternoon). Venepuncture of the left antecubital vein was performed with tourniquet. Blood was collected into plain and fluoride Sarstedt (Neumbrecht, Germany) monovettes. Serum for lipid analyses was refrigerated at −4°C and assayed within 72 hours (22). Total cholesterol was determined by an enzymatic procedure using the CHOD-PAP method at phases 3 and 7. Serum HDL-cholesterol concentrations were measured from the supernatant after precipitation of non-HDL-cholesterol with dextran sulphate-magnesium at phase 3 and with a direct homogeneous assay at phase 7 (23), using at both phases the CHOD-PAP method. Serum triglyceride was determined by enzymatic colorimetric method (GPO-PAP) at both phases. The concentration of LDL-cholesterol was calculated using the Friedewald formula when serum triglycerides were lower than 4.5 mmol/L (24). Technical error was estimated by assaying blinded duplicate samples for 5% of subjects. Coefficients of variation were 2.0% to 6.6%. After both screenings, participants were sent a letter which informed them of their results and summarised whether or not they were "at increased risk of heart disease or angina". For example, when a total cholesterol level of 8.5 mmol/L or higher was recorded at baseline (n=185), the letter suggested the participant see his or her GP for a repeat test. The same envelope contained a similar unsealed letter addressed to the participant's GP.

Potential predictors

Lipid lowering drugs

At phases 3 and 7, participants were asked whether they had taken any medication in the last 14 days and, if so, to provide the name of the medication. Medications were coded using British National Formulary codes which do not distinguish statins from other lipid lowering drugs, such

as fibrates, nicotinic acid and its derivatives, cholesterol absorption inhibitors, or omega-3 fatty acid compounds. Thus, our measure included all lipid lowering drugs combined together.

Diet quality using the Alternate Healthy Eating Index (AHEI)

Diet quality was measured using the AHEI. (25). Based on the 127 item anglicized version of Willett's Food Frequency Questionnaire (FFQ) (26, 27), it has been found to yield a satisfactory estimate of food intake among Whitehall II participants compared to biomarkers and 7-day diet diaries (27). The AHEI includes nine food components; food items listed on the FFQ were assigned to their appropriate food groups, using the FFQ serving sizes identified. Eight of the nine components (vegetables, fruit, nuts and soy protein, ratio of white to red meat, dietary fibre, trans fat, ratio of polyunsaturated to saturated fatty acids, and alcohol) contributed 0-10 points to the total AHEI score. A score of 10 indicates that recommendations were fully met, whereas a score of 0 represents the least healthy dietary behaviour. Intermediate intakes were scored proportionally between 0 and 10. The final component, long-term multivitamin use, was dichotomized, contributing either 2.5 points (for non-use) or 7.5 points (for use) to the total score. All component scores were summed to obtain a total AHEI score ranging from 2.5 (worst) to 87.5 (best) (25). Nutrient intake estimates were calculated using a computerized system developed for the Whitehall II dietary data (27).

Physical activity

At baseline (phase 3), participants were asked the duration (number of hours per week) of their participation in moderately energetic (*e.g.*, dancing, cycling, leisurely swimming, lawn mowing), and vigorous (*e.g.*, running, hard swimming, playing squash) physical activity. At phase 7, the questionnaire was modified to include 20 items on duration of participation in different physical

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activities (*e.g.*, running, cycling, other sports, housework, and gardening activities) that were used to compute hours per week at each intensity level. At both phases, physical activity was defined as the total number of hours per week spent in moderate and vigorous activity.

Covariates

Other variables included in the analysis were: sex; age at baseline (<45, 45-54, ≥55 years); self-reported ethnicity (White, non-White); education (none, lower secondary, A-levels, university or higher); current smoking (categorized as yes, no); and longstanding illness (categorized as yes, no). Prevalent CHD was defined using the MONICA criteria (28), positive responses to questions about chest pain (29) and physician diagnoses, evidence from medical records, or ECG findings. Prevalent diabetes mellitus was defined as reported doctor-diagnosed diabetes mellitus or use of diabetes medication (30). Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared and categorized as normal (BMI<25), overweight (25≤BMI <30), or obese (≥30) (31).

Statistical analyses

Eleven-year change in LDL-cholesterol was calculated as phase 7 minus phase 3 values. In the analysis we wanted to determine the impact of the predictors assessed at baseline (phase 3) and their values over the 11-year follow-up. In order to simplify the interpretation of the coefficients, we categorized the predictors in the following way: lipid lowering drug use was categorized as no use, treated at baseline, or treatment started during the follow-up. Change in diet was estimated by subtracting AHEI score at baseline from that at phase 7, standardized to a z-score (mean=0, standard deviation (SD)=1) and categorized as "increase" (z-score≥1), "stable" (-1≤z-score<1), and "decrease" (z-score≤-1). This procedure was first applied to the total AHEI score and then to

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the 9 components of the score. Change in physical activity was calculated by subtracting the number of hours per week of physical activity at baseline from that at phase 7. The difference in duration was standardized and categorized as an "increase" (z-score≥1), "stable" (-1≤z-score<1), "decrease" (z-score≤-1) level of physical activity.

As 11-year changes in LDL-cholesterol, diet score, and physical activity were all normally distributed, parametric statistical tests were used in the analysis. To examine the unadjusted impact of the predictors (lipid lowering drug, diet, and physical activity) and the covariates we first conducted univariate analysis using linear regression with change in LDL-cholesterol as the dependent variable. The interaction terms between the predictors and sex and age had p values $p>0.05$ negating any necessity to stratify the analyses by age or sex. For the quantitative predictors (diet and physical activity) and for change in BMI, we tested the interaction between continuous values at baseline and the change measures expressed as "increase", "stable", or "decrease". Only the interaction term between BMI at baseline and change in BMI was significant ($p<0.05$) and consequently was included in further analyses as a covariate.

We constructed 3 models to examine associations between the predictors and concomitant change in LDL-cholesterol. Model 1 included only non-modifiable covariates (sex, age at baseline, ethnicity) and the duration of follow-up. Model 2 further included education, BMI, and longstanding illness at baseline. Model 3 included all three predictors together with the covariates already in model 2. To examine the role of regression to the mean in declining LDL-cholesterol trends, we repeated Model 3 among participants in the highest quintile of LDL-cholesterol at baseline (in this subgroup regression to the mean is particularly likely) (32), and compared the results to those from the main analysis. In addition, for lipid lowering medication we removed any potential regression to the mean effect by comparing those with lipid-lowering

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medication during follow-up with a group without medication selected such that their mean LDL-cholesterol values at baseline were identical.

Using the covariates and predictors in model 3, we conducted several supplementary analyses to determine: 1) whether specific components of the 9 item AHEI scale were associated with the reduction in LDL-cholesterol; 2) the effect of moderate and vigorous physical activity separately on change in LDL-cholesterol; 3) the impact of further adjusting model 3 for change in BMI and the interaction term between change in BMI and BMI at baseline; and 4) whether replacing the variable "presence of a long standing illness at baseline" by the variables "presence of at least one non-fatal CHD event at baseline" and "presence of diabetes mellitus at baseline" changed estimates.

The squared multiple correlation, also called coefficient of determination (R^2), was used to estimate the proportion of variation in LDL-cholesterol change explained by the predictors. We assessed change in R^2 when each predictor was entered individually into: 1) the initial model adjusted for sex, age at baseline, ethnicity, duration of follow-up, education level, BMI at baseline, and longstanding illness at baseline; 2) the model above plus mutual adjustment for the predictors (33).

To examine potential beneficial effects related to lipid lowering drug treatment and favourable changes in diet and physical activity, we estimated the reduction in LDL-cholesterol that would be observed if 1) all participants in need of lipid-lowering drugs at baseline (n=858) were treated, 2) all individuals with a non-optimal diet (AHEI score <60, n=3457) improved their diet, defined as an increased AHEI score of at least 1 SD (0.6 point), and 3) those who undertake less than the recommended level of physical activity (<2.5 hours per week, n=2190) increased their physical activity, defined as a minimum increase of 17 minutes (1 SD) physical activity per week. Following European guidelines (20), participants with prevalent CHD or diabetes and

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those with a "high risk" of cardiovascular disease (CVD) defined as having 10-year risk of CVD death of 5% or more based on the SCORE (Systematic COronary Risk Evaluation) charts (34), were deemed to be in need of lipid lowering therapy. The benefits of lipid-lowering treatment, improved diet (≥ 1 SD) and increased physical activity levels (≥ 1 SD) for the total population were estimated based on Model 3 estimates using the following equation:

$$\hat{y} = \text{Intercept} + \beta 1\ I_{\text{treatment at baseline}} + \beta 2\ I_{\text{treatment during the follow-up}} + \beta 3\ I_{\text{one or more SD decrease in AHEI score}} + \beta 4\ I_{\text{one or more SD increase in AHEI score}} + \beta 5\ I_{\text{one or more SD decrease in hours of physical activity}} + \beta 6\ I_{\text{one or more SD increase in hours of physical activity}} + \beta 7\ \text{Men} + \beta 8\ \text{Age at baseline} + \beta 9\ \text{Ethnicity} + \beta 7\ I_{\text{intermediate education}} + \beta 8\ I_{\text{high education}} + \beta 9\ \text{BMI at baseline} + \beta 10\ \text{Longstanding illness} + \beta 11\ \text{Duration of follow-up},$$

where $\hat{y}$ is change in LDL cholesterol and I an indicator variable (1 vs 0). All analyses were performed with SAS software, version 9 (SAS Institute Inc, USA).

RESULTS

Description of the study participants

Of the 10,308 participants at recruitment to the study, 7,583 had complete data at phase 3 and 4,469 were included in the analysis reported here (**Figure 1**, comparison of the participants included in the analyses to those excluded is provided in **Appendix, eTable 1**). **Table 1** describes the characteristics of those included at baseline and follow-up. The mean age at baseline was 49.3 years and 72.0% were men. From baseline to follow-up, the mean LDL-cholesterol concentration dropped from 4.38 to 3.52 mmol/L. At the same time the use of lipid lowering drugs increased from 0.8% to 10.8%. There was a small increase in the mean total AHEI score (from 50.7 to 51.2) and the mean number of hours/week spent in moderate or vigorous physical activity (from 3.4 to 3.7 hours/week).

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At baseline, 858 participants had a high risk of CVD according to the European guidelines (20), had diabetes or had experienced a validated non-fatal CHD event at baseline, or took lipid lowering medication at baseline or follow-up. Only 60.0% (n=515) of them were taking lipid lowering medication at follow-up.

Multivariate analysis of change in lipid levels during the follow-up

Univariate analyses provided strong evidence of associations between changes in LDL-cholesterol and all the covariates, with the exception of smoking (**Appendix eTable 2)**. **Table 2** presents multivariable-adjusted absolute changes in LDL-cholesterol as a function of lipid lowering medication and changes in diet and physical activity. These results show that LDL-cholesterol declined in all groups. **Table 3** shows the corresponding changes in relative terms. Compared to those not on lipid lowering drugs, decline in LDL-cholesterol was greater among those who were on treatment at baseline or during the follow-up. Compared to those with a stable diet, individuals who improved their diet showed greater decline in LDL-cholesterol while those whose diet worsened showed a smaller decline. Similar results were observed for physical activity. All relative differences persisted after serial adjustments for multiple covariates (Table 3). The results were largely similar in a sub-group of participants in the top quintile of LDL-cholesterol at baseline and when, for lipid lowering medication, potential regression to the mean effects were totally removed (**Appendix eTable 3**).

More detailed analyses of the 9 components of the AHEI diet score and intensity of physical activity are shown in **Appendix eTables 4 and 5**. Briefly, the decline of LDL-cholesterol change was significantly associated with an increase of the ratio of white to red meat consumption ($p<0.001$), the ratio of polyunsaturated to saturated fatty acids ($p<0.001$), increase in

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the fruit consumption (p=0.04) and decrease in trans fats (p=0.04). Decreases in both moderate and vigorous physical activity were associated with a smaller decrease in LDL-cholesterol.

In sensitivity analyses, we examined the role of BMI by adding the following covariates to model 3: change in BMI and the interaction term between change in BMI and BMI at baseline. The results remained largely unchanged. Similarly, when the variable "presence of a long standing illness at baseline" was replaced by the variables "presence of at least one non-fatal CHD event at baseline" and "presence of diabetes mellitus at baseline" respectively in Model 3, the results were much the same (data not shown).

Multivariate analyses of changes in other lipid fractions are provided in **Appendix, eTable 6.** An increase in physical activity was associated with a 0.05 mmol/L greater increase in HDL-cholesterol compared with those who had a stable level of physical activity. Participants whose BMI increased over the follow-up showed a 0.01 mmol/L decrease in HDL-cholesterol and 0.27 mmol/L increase in triglycerides; among those with stable BMI, HDL-cholesterol increased by 0.11 mmol/L and triglycerides decreased by 0.07 mmol/L. Participants whose alcohol consumption increased had a 0.08 mmol/L greater increase in HDL-cholesterol than those whose alcohol consumption was stable. Among participants who stopped smoking during follow-up, there was a 0.04 mmol/L greater increase in HDL-cholesterol compared with never smokers.

Relative importance of medications, diet and physical activity in explaining LDL-cholesterol trends

The baseline model for this analysis, including sex, ethnicity, duration of follow-up and baseline measures of age, education, BMI and longstanding illness as covariates, explained 11.6% of the variability in the change in LDL-cholesterol. Adding lipid lowering drugs to this model increased the coefficient of determination (R^2) by 29.4%. AHEI diet score and physical activity, when

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added into the baseline model, explained only 0.5% and 0.3% of the variability in the change in LDL-cholesterol concentrations, respectively. Each predictor had an independent effect in the mutually adjusted model. (**Appendix eTable 7**)

Estimated population-level benefits of lipid-lowering drugs and improved lifestyle

Based on Model 3 estimates, if all 858 participants with prevalent CHD or diabetes or a high risk of CVD death at baseline had been on lipid-lowering medication, as suggested in European guidelines, then the decline in LDL-cholesterol would have been 2.77 mmol/L greater than the observed value (**Table 4**). If all 3457 participants who did not have an optimal diet (AHEI score <60) had improved their diet, the corresponding additional decline in LDL-cholesterol would have been 0.08 mmol/L. Adoption of a more physically active lifestyle by the 2190 participants who undertook less than 2.5 hours of moderate or vigorous activities per week would have produced an additional decline in LDL-cholesterol of 0.11 mmol/L. These estimations applied to the total cohort (n=4469) suggest that successful implementation of lipid-lowering therapy and change in lifestyle would each reduce LDL-cholesterol levels by 0.90 to 1.07 mmol/L (**Table 4**).

DISCUSSION

We found an overall decrease in LDL-cholesterol concentration in the Whitehall II cohort of civil servants over 11 years of follow-up. The degree of decline was associated with an increased use of lipid lowering drugs, improvements in diet – especially the ratio of white to red meat consumption and the ratio of polyunsaturated to saturated fatty acids intake – and increase in physical activity. In this population, the contribution of changes in diet and physical activity were

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modest compared to pharmacological treatment among individuals at high risk of CVD. However, a successful implementation of lipid-lowering drug treatment for the relatively small group of high-risk individuals and a favourable change in diet and physical activity in the large group of people with a non-optimal lifestyle were estimated to result in largely similar declines in LDL-cholesterol in the total cohort. These findings support the use of multifaceted intervention strategies for prevention.

In many previous studies, decrease in LDL-cholesterol concentration has been assessed by comparing cross-sectional surveys repeated over time: in the INTERGENE and GOT-MONICA study (1985, 1990, 1995, and 2002) (9), in the French MONICA study (1996 and 2007) (10), in the studies conducted in Catalonia, Spain (1992 and 2003) (11) and in Gerona, Spain (1995, 2000, and 2005) (12). This design captures time trends but, unlike the prospective cohort design employed in the present study, does not allow estimation of within-subject changes in LDL-cholesterol, or in their predictors.

Our study confirms the findings of the few prior cohort studies on changes in total or LDL-cholesterol among middle-aged individuals. In an Australian population-based cohort study, Buyken et al (35) reported a decrease of 0.7 mmol/L in LDL-cholesterol between 1992 and 2004, comparable to the 0.9 mmol/L decrease in our study. Two other cohort studies, the New Zealand Workforce Diabetes Survey (19) and the American Physicians' Health Study (36), also reported a decline in LDL-cholesterol from 1988 to 1997 and from 1982 to 1997, respectively. In the Framingham Heart Study (37), there was a slight increase in LDL-cholesterol over time, but these analyses did not include individuals on lipid lowering or hormone replacement therapies, or those with prevalent cardiovascular disease. Randomized trials have shown lipid lowering drugs (38, 39), diet modification (39-42) and endurance exercise training (42-44) to be effective in lowering LDL-cholesterol concentrations. The present results, obtained from an observational study, add to

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knowledge from randomized controlled trials where the effect size is dependent on specific interventions.

There are a few caveats to the results reported here. First, total cholesterol and triglyceride were not measured using the same enzymatic methods at both study phases; but HDL-cholesterol was assessed using the dextran sulphate-magnesium precipitation method at baseline and the direct homogeneous method at follow-up (23). These protocol changes might have affected the estimation of absolute LDL levels. However, this is an unlikely source of major bias because both methods have been validated and certified by the Cholesterol Reference Method Laboratory Network at the Centers for Disease Control and Prevention (45, 46), and the agreement between the methods is high with a correlation coefficient of 0.98, slope 0.98 and mean bias 0.05 mmol/L (47). If the level of HDL-cholesterol was "overestimated" by 0.05 mmol/L at follow-up in the present study, the method-related decrease in LDL-cholesterol between baseline and follow-up would have been approximately 0.06 mmol/L, which is small compared to the mean observed decrease of 0.86 mmol/L. Furthermore, bias resulting from the change in the method of assessing HDL-cholesterol is likely to be independent of the measurement of the predictors and thus should not unduly bias our findings on relative differences in changes in LDL-cholesterol between subgroups.

Second, physical activity and, to a greater extent, dietary intake, are difficult to measure accurately; while it is likely that use of lipid-lowering drugs is recalled with greater precision. We may have therefore underestimated the effects of diet and physical activity on LDL-cholesterol decline. Furthermore, it is possible that we underestimated the contribution of diet because our analysis did not fully capture effects arising from externally driven secular changes in dietary patterns. For example, recommendations from the National Institute for health and Clinical Excellence (NICE) encourage manufacturers, caterers and producers to reduce the amount of

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saturated and trans fatty acids in all food products and replace them, if needed, by polyunsaturated and monounsaturated fatty acids (48). Such guidance, if successful in reducing "bad" cholesterol in marketed foods, could, potentially, have a notable impact on the number of cardiovascular events at the population level, as is clear from results from the recent meta-analysis of randomised controlled trials (49).

Third, regression toward the mean is a potential source of bias in observational studies with repeat outcome measures (32). Regression to the mean arises from random errors in measurement and should be relatively independent of the use of lipid-lowering drugs or lifestyle. In the present study, these factors remained important predictors of reduced LDL levels in a subgroup of participants with particularly high LDL-cholesterol at baseline, a group whose measures are likely to contain more measurement error, suggesting that regression to the mean had, if anything, little impact on our findings.

Fourth, by definition, occupational cohorts such as Whitehall II are fitter than the general population due to the healthy worker effect. The feedback provided to participants after medical screening Phases of the study about their coronary risk factors may also have promoted healthier lifestyles, as discussed in relation to the Framingham study (37). Thus, further research is needed to examine the generalisability of our findings.

With the limitations of our study in mind, we conclude that declining trends in LDL-cholesterol seems to be independently associated with the use of lipid lowering therapy and favourable lifestyle changes. Our findings suggest that more should be done to reduce under-treatment of dyslipidaemia and promote lifestyle modifications in order to further accelerate the favourable population trends in LDL-cholesterol.

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Table 1. Baseline (phase 3) and follow-up (phase 7) characteristics of the 4,469 study participants.

	Baseline (1991-93)		Phase 7 (2003-04)	
	N	% / Mean (SD)	N	% / Mean (SD)
Sex				
Men	3217	72.0	-	-
Women	1252	28.0	-	-
Age (years)				
<45	1198	26.8	-	-
[45-55[	2169	48.5	-	-
≥55	1102	24.7	-	-
All	4469	49.3 (5.9)	-	-
Ethnicity				
White	4189	93.7	-	-
Non-White	280	6.3	-	-
Education				
No or lower secondary	1969	44.1	-	-
A levels	1171	26.2	-	-
University or higher	1329	29.7	-	-
BMI (kg/m^2)				
Normal (<25)	2463	55.1	1718	38.4
Overweight ([25-30[)	1667	37.3	1999	44.7
Obese (≥30)	339	7.6	752	16.8
All	4469	25.0 (3.5)	4469	26.5 (4.2)
Current smoking				
No	4000	89.5	4159	93.1
Yes	469	10.5	310	6.9

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Longstanding illness				
No	2993	67.0	1767	39.5
Yes	1476	33.0	2702	60.5
Total cholesterol concentrations (mmol/L)	4469	6.4 (1.1)	4469	5.7 (1.0)
HDL-cholesterol concentrations (mmol/L)	4469	1.4 (0.4)	4469	1.6 (0.4)
Triglyceride concentrations (mmol/L)	4469	1.3 (0.7)	4469	1.3 (0.7)
LDL-cholesterol concentrations (mmol/L)	4469	4.4 (1.0)	4469	3.5 (0.9)
Lipid lowering drugs use				
No	4435	99.2	3987	89.2
Yes	34	0.8	482	10.8
AHEI score, mean (SD)	4469	50.7 (11.9)	4469	51.2 (12.5)
Physical activity (hours/week)	4469	3.4 (3.4)	4469	3.7 (3.1)

SD = standard deviation; LDL = low-density lipoprotein; BMI = body mass index; AHEI = alternate healthy eating index.

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Table 2. Absolute change in serum LDL-cholesterol between the baseline (1991-1993) and follow-up (2003-2004) screening as function of use of lipid-lowering drugs, healthy diet, and physical activity, (N=4,469).

		Mean absolute change in LDL-cholesterol (95% CI), mmol/L		
	n	Model 1*	Model 2†	Model 3‡
Start of lipid lowering drug				
None	3954	-0.61 (-0.66 to -0.56)	-0.60 (-0.65 to -0.56)	-0.59 (-0.65 to -0.53)
Baseline	34	-1.70 (-1.96 to -1.43)	-1.70 (-1.96 to -1.43)	-1.73 (-1.99 to -1.46)
During follow-up	481	-2.42 (-2.50 to -2.34)	-2.38 (-2.46 to -2.30)	-2.36 (-2.45 to -2.28)
Change in AHEI score				
Increase (≥1 SD)	717	-0.99 (-1.08 to -0.91)	-0.99 (-1.07 to -0.90)	-1.65 (-1.76 to -1.53)
Stable (-1≤SD<1)	3071	-0.85 (-0.92 to -0.79)	-0.84 (-0.90 to -0.78)	-1.58 (-1.68 to -1.47)
Decrease (<-1 SD)	681	-0.76 (-0.85 to -0.67)	-0.75 (-0.83 to -0.66)	-1.46 (-1.57 to -1.35)
Change in physical activity				
Increase (≥1 SD)	601	-0.98 (-1.07 to -0.88)	-0.95 (-1.05 to -0.86)	-1.66 (-1.78 to -1.54)
Stable (-1≤SD<1)	3312	-0.86 (-0.92 to -0.80)	-0.85 (-0.91 to -0.79)	-1.56 (-1.66 to -1.46)
Decrease (<-1 SD)	556	-0.77 (-0.87 to -0.68)	-0.76 (-0.85 to -0.66)	-1.46 (-1.58 to -1.34)

CI = confidence interval; other abbreviations as in Table 1.

* Model 1: Adjusted for sex, age at baseline, ethnicity, and duration of follow-up.

† Model 2: As model 1 and additionally adjusted for education level, BMI at baseline, and longstanding illness at baseline.

‡ Model 3: As model 2 with predictors mutually adjusted.

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Table 3. Relative change in serum LDL-cholesterol (mmol/L) between the baseline (1991-1993) and follow-up (2003-2004) screening as a function of use of lipid-lowering drugs, healthy diet, and physical activity, (N=4,469).

		Mean relative change in LDL-cholesterol (mmol/L) and P-value for difference					
	n	Model 1*		Model 2†		Model 3‡	
Start of lipid lowering drug							
None	3954	0	Reference	0	Reference	0	Reference
Baseline	34	-1.09	<0.001	-1.09	<0.001	-1.14	<0.001
During follow-up	481	-1.81	<0.001	-1.77	<0.001	-1.77	<0.001
Change in AHEI score							
Increase (≥1 SD)	717	-0.14	<0.001	-0.14	<0.001	-0.07	0.03
Stable (-1≤SD<1)	3071	0	Reference	0	Reference	0	Reference
Decrease (<-1 SD)	681	0.10	0.02	0.10	0.02	0.12	<0.001
Change in physical activity							
Increase (≥1 SD)	601	-0.11	0.007	-0.10	0.02	-0.10	0.005
Stable (-1≤SD<1)	3312	0	Reference	0	Reference	0	Reference
Decrease (<-1 SD)	556	0.09	0.04	0.09	0.03	0.10	0.004

Abbreviations as in Tables 1 and 2.

* Model 1: Adjusted for sex, age at baseline, ethnicity, and duration of follow-up.

† Model 2: As model 1 and additionally adjusted for education level, BMI at baseline, and longstanding illness at baseline.

‡ Model 3: As model 2 with predictors mutually adjusted.

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Table 4. Estimated beneficial effect of lipid lowering drugs, healthy diet, and physical activity on LDL-change in the population at risk and the total cohort

Intervention	Mean LDL-cholesterol (mmol/L) change				
	Population at risk at baseline			Total cohort (n=4469)	
	Total N (N already following the intervention)*	Observed	After intervention†	Observed	After intervention†
Start lipid lowering drugs	858‡ (515)	-1.04	-3.81	-0.86	-1.07
≥1 SD increase in the AHEI diet score*	3457§ (684)	-0.84	-0.92	-0.86	-0.91
≥1 SD increase in the number of hours of physical activity*	2190# (383)	-0.85	-0.96	-0.86	-0.90

* Here intervention stands for use of a lipid lowering drug among those needed such a treatment according to the European guidelines, improving diet among those with AHEI score <60, or increasing duration of physical activity among those with <2.5 hours per week. 1 SD increase in AHEI score is 0.6 point and 1 SD increase in physical activity is 17 minutes per week

† Decline in LDL-cholesterol estimated for participants who met the criteria for intervention based on effects shown in Table 3, Model 3.

‡ Participants with CVD risk score ≥ 5% or prevalent CHD or diabetes at baseline, or lipid lowering medication at baseline or follow-up.

§ Participants with AHEI score <60 at baseline.

Participants with physical activity<2.5 hours/week at baseline.

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FIGURE LEGENDS

Figure 1. Selection of the study participants.

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Appendices

eTable 1. Comparison of characteristics between participants included in the present analysis and participants not included but eligible at baseline (phase 3), N=7,583.

	Study participants N=4,469		Not included participants N=3,114		P-value
	N	% / Mean (SD)	N	% / Mean (SD)	
Sex					
Men	3217	72.0	2014	64.7	<0.001*
Women	1252	28.0	1100	35.3	
Age (years)					
<45	1198	26.8	767	24.6	<0.001†
[45-55[	2169	48.5	1438	46.2	
≥55	1102	24.7	909	29.2	
All	4469	49.3 (5.9)	3114	49.9 (6.2)	<0.001‡
Ethnicity					
White	4189	93.7	2661	85.5	<0.001*
Non-White	280	6.3	453	14.6	
Missing data	-	-	*2173*	*69.8*	-
Education					
No or lower secondary	1969	44.1	1269	49.1	<0.001†
A levels	1171	26.2	638	24.7	
University or higher	1329	29.7	677	26.2	
Missing data	-	-	*530*	*17.0*	-
BMI (kg/m^2)					
Normal (<25)	2463	55.1	1563	50.2	<0.001†
Overweight ([25-30[)	1667	37.3	1181	37.9	
Obese (≥30)	339	7.6	370	11.9	

All	4469	25.0 (3.5)	3114	25.5 (3.9)	<0.001‡
Current smoking					
No	4000	89.5	2575	82.7	<0.001*
Yes	469	10.5	539	17.3	
Longstanding illness					
No	2993	67.0	2036	65.4	0.15*
Yes	1476	33.0	1078	34.6	
LDL-cholesterol concentrations (mmol/L)	4469	4.4 (1.0)	3114	4.4 (1.1)	0.08‡
Lipid lowering drugs use					
No	4435	99.2	3093	99.3	0.66*
Yes	34	0.8	21	0.7	
AHEI score, mean (SD)	4469	50.7 (11.9)	3114	49.6 (12.8)	<0.001‡
Physical activity (hours/week)	4469	3.4 (3.4)	3114	3.2 (3.4)	0.002‡

SD = standard deviation; LDL = low-density lipoprotein; BMI = body mass index; AHEI = alternate healthy eating index.

* Chi-square test; † Cochrane-Armitage trend test; ‡ Student's t-test.

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eTable 2. Univariate association between the 11-year serum LDL-cholesterol change (mmol/L) and covariates (n=4,469).

	N (%)	LDL-cholesterol mean change in mmol/L (SD)	P-value*
Sex			
Male	3217 (71.98)	-0.93 (0.97)	Reference
Female	1252 (28.02)	-0.66 (1.05)	<0.001
Age at baseline (years)			
<45	1198 (26.81)	-0.55 (0.85)	Reference
[45-55[	2169 (48.53)	-0.84 (0.96)	<0.001
≥55	1102 (24.66)	-1.23 (1.10)	<0.001
Ethnicity			
White	4189 (93.73)	-0.85 (0.99)	0.02
Non-White	280 (6.27)	-1.00 (1.13)	Reference
Education			
No or lower secondary	1969 (44.06)	-0.93 (1.06)	Reference
A levels	1171 (26.20)	-0.82 (0.95)	0.004
University or higher	1329 (29.74)	-0.79 (0.95)	<0.001
BMI at baseline (kg/m^2)			
Normal (<25)	2463 (55.11)	-0.72 (0.95)	Reference
Overweight ([25-30[)	1667 (37.30)	-1.01 (1.02)	<0.001
Obese (≥30)	339 (7.59)	-1.10 (1.08)	<0.001
Change in BMI			
Increase (≥1 SD)	566 (12.67)	-0.58 (1.08)	<0.001
Stable (-1≤SD<1)	3367 (75.34)	-0.85 (0.97)	Reference
Decrease (<-1 SD)	536 (11.99)	-1.18 (0.99)	<0.001
Current smoking at baseline			
No	4000 (89.51)	-0.87 (1.00)	Reference

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Yes	469 (10.49)	-0.86 (1.00)	0.80
Longstanding illness at baseline			
No	2993 (66.97)	-0.81 (0.96)	Reference
Yes	1476 (33.03)	-0.95 (1.07)	<0.001
CHD at baseline			
No	4368 (97.74)	-0.84 (0.99)	Reference
Yes	101 (2.26)	-1.64 (1.22)	<0.001
Diabetes at baseline			
No	4379 (97.99)	-0.85 (0.99)	Reference
Yes	90 (2.01)	-1.35 (1.16)	<0.001
Start of lipid lowering drug			
None	3954 (88.48)	-0.64 (0.75)	Reference
Baseline	34 (0.76)	-1.88 (1.52)	<0.001
Follow-up	481 (10.76)	-2.55 (1.07)	<0.001
AHEI score at baseline			
≤51.5 points	2361 (52.83)	-0.86 (0.96)	Reference
>51.5 points	2108 (47.17)	-0.85 (1.04)	0.75
Change in AHEI score			
Increase (≥1 SD)	717 (16.04)	-0.99 (1.05)	<0.001
Stable (-1≤SD<1)	3071 (68.72)	-0.85 (0.98)	Reference
Decrease (<-1 SD)	681 (15.24)	-0.77 (1.00)	0.06
Physical activity (hours/week) at baseline			
≤2.5	2295 (51.35)	-0.87 (1.00)	Reference
>2.5	2174 (48.65)	-0.84 (0.99)	0.30
Change in physical activity			
Increase (≥1 SD)	601 (13.45)	-1.03 (1.11)	<0.001
Stable (-1≤SD<1)	3312 (74.11)	-0.84 (0.97)	Reference
Decrease (<-1 SD)	556 (12.44)	-0.77 (1.01)	0.16

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Duration of follow-up (year)	4469 (100.00)	-0.16 (0.03)	<0.001

LDL = low-density lipoprotein; SD = standard deviation; BMI = body mass index; CHD = coronary heart disease; AHEI = alternate healthy eating index. *P-value for relative difference.

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eTable 3. Absolute change* in serum LDL-cholesterol between baseline (1991-1993) and follow-up (1991-1993) screenings according to different predictors among those who were in the highest quintile of LDL-cholesterol at baseline (n=899).

	n	Mean absolute change* in LDL-cholesterol, 95% CI, mmol/L	P-value†
Start of lipid lowering drug‡			
None	637	-1.28 (-1.42 to -1.14)	Reference
Baseline	17	-2.77 (-3.19 to -2.36)	<0.0001
During follow-up	245	-3.02 (-3.18 to -2.87)	<0.0001
Change in AHEI score			
Increase (≥1 SD)	162	-2.51 (-2.73 to -2.29)	0.15
Stable (-1≤SD<1)	610	-2.41 (-2.59 to -2.22)	Reference
Decrease (<-1 SD)	127	-2.16 (-2.38 to -1.94)	0.002
Change in physical activity			
Increase (≥1 SD)	142	-2.52 (-2.74 to -2.30)	0.01
Stable (-1≤SD<1)	651	-2.33 (-2.51 to -2.15)	Reference
Decrease (<-1 SD)	106	-2.23 (-2.46 to -2.00)	0.24

LDL = low-density lipoprotein; CI = confidence interval; SD = standard deviation.

* Adjusted for sex, age at baseline, ethnicity, duration of follow-up, education level, BMI at baseline and longstanding illness at baseline, lipid lowering drug use, and diet score.

† P-value for relative difference.

‡ In a supplementary analysis, the mean decline in LDL cholesterol among the 481 participants with lipid-lowering medication during the follow-up was 2.48 mmol/L compared to 1.01 mmol/L in a subgroup of 1459 non-users with high LDL (selected because their mean at baseline was the same as in the lipid-lowering medication group) (p<0.0001).

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eTable 4. Absolute change in serum LDL-cholesterol between the baseline (1991-1993) and follow-up (2003-2004) screenings as a function of change in each component of AHEI (n=4,469).

AHEI score change	Mean absolute change* in LDL-cholesterol (95% CI), mmol/L	P-value†
Vegetables		
Increase (≥1 SD)	-1.53 (-1.64 to -1.41)	0.34
Stable (-1≤SD<1)	-1.56 (-1.66 to -1.45)	Reference
Decrease (<-1 SD)	-1.60 (-1.71 to -1.48)	0.27
Fruits		
Increase (≥1 SD)	-1.62 (-1.73 to -1.50)	0.04
Stable (-1≤SD<1)	-1.55 (-1.65 to -1.45)	Reference
Decrease (<-1 SD)	-1.54 (-1.65 to -1.42)	0.66
Nuts and soy protein		
Increase (≥1 SD)	-1.53 (-1.66 to -1.41)	0.37
Stable (-1≤SD<1)	-1.57 (-1.67 to -1.46)	Reference
Decrease (<-1 SD)	-1.52 (-1.64 to -1.39)	0.20
Ratio of white to red meat		
Increase (≥1 SD)	-1.67 (-1.79 to -1.56)	<0.001
Stable (-1≤SD<1)	-1.55 (-1.65 to -1.45)	Reference
Decrease (<-1 SD)	-1.51 (-1.62 to -1.39)	0.17
Dietary fibre		
Increase (≥1 SD)	-1.60 (-1.72 to -1.49)	0.14
Stable (-1≤SD<1)	-1.55 (-1.66 to -1.45)	Reference
Decrease (<-1 SD)	-1.54 (-1.66 to -1.42)	0.67
Trans fat		
Increase (≥1 SD)	-1.58 (-1.70 to -1.46)	0.57
Stable (-1≤SD<1)	-1.56 (-1.67 to -1.46)	Reference
Decrease (<-1 SD)‡	-1.48 (-1.60 to -1.36)	0.02

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PUFAs/SFAs		
Increase (≥1 SD)	-1.69 (-1.80 to -1.57)	<0.001
Stable (-1≤SD<1)	-1.56 (-1.66 to -1.45)	Reference
Decrease (<-1 SD)	-1.45 (-1.56 to -1.34)	<0.001
Duration of multivitamin use		
Increase (≥1 SD)	-1.52 (-1.64 to -1.40)	0.17
Stable (-1≤SD<1)	-1.57 (-1.68 to -1.47)	Reference
Decrease (<-1 SD)	-1.55 (-1.66 to -1.43)	0.42
Alcohol		
Increase (≥1 SD)	-1.55 (-1.67 to -1.43)	0.61
Stable (-1≤SD<1)	-1.57 (-1.67 to -1.46)	Reference
Decrease (<-1 SD)§	-1.47 (-1.60 to -1.35)	0.01

AHEI = alternate healthy eating index; LDL = low-density lipoprotein; CI = confidence interval; SD = standard deviation; PUFAs/SFAs = ratio of polyunsaturated to saturated fatty acids.

* Adjusted for sex, age at baseline, ethnicity, duration of follow-up, education level, BMI at baseline, and having a longstanding illness at baseline, lipid lowering drug use, and physical activity.

† P-value for relative difference.

‡ "Decrease" in trans fat score corresponds to an increased consumption of trans fatty acids during follow-up.

§ "Decrease" in alcohol score corresponds to a "non-ideal" consumption of alcohol during follow-up.

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eTable 5. Absolute change in serum LDL-cholesterol between the baseline (1991-1993) and follow-up (2003-2004) screenings as a function of change in physical activity (hours/week) and intensity of physical activity. (n=4,469).

	Moderate*		Vigorous†		Moderate and vigorous	
	Mean absolute change‡ in LDL-cholesterol, 95% CI, mmol/L	P-value§	Mean absolute change‡ in LDL-cholesterol, 95% CI, mmol/L	P-value§	Mean absolute change‡ in LDL-cholesterol, 95% CI, mmol/L	P-value§
Change in physical activity						
Increase (≥1 SD)	-1.60 (-1.71 to -1.48)	0.36	-1.58 (-1.70 to -1.46)	0.75	-1.66 (-1.78 to -1.54)	0.005
Stable (-1≤SD<1)	-1.57 (-1.67 to -1.46)	Reference	-1.56 (-1.66 to -1.46)	Reference	-1.56 (-1.66 to -1.46)	Referenc
Decrease (<-1 SD)	-1.47 (-1.59 to -1.35)	0.008	-1.50 (-1.61 to -1.38)	0.04	-1.46 (-1.58 to -1.34)	0.004

LDL = low-density lipoprotein; CI = confidence interval; SD = standard deviation.

* Moderate physical activity included sports such as dancing, swimming.

† Vigorous physical activity included sports such as running, playing squash.

‡ Adjusted for sex, age at baseline, ethnicity, duration of follow-up, education level, BMI at baseline, and having a longstanding illness at baseline, lipid lowering drug use, and diet score.

§ P-value for relative difference.

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eTable 6. Absolute change* in serum HDL-cholesterol and triglyceride between the baseline (1991-1993) and follow-up (2003-2004) screenings according to different predictors (n=4,469)

	n	Mean absolute change* in HDL-cholesterol (95% CI) mmol/L	P-value†	Mean absolute change* in triglyceride (95% CI) mmol/L	P-value†
Start of lipid lowering drug					
None	3954	0.13 (0.10 to 0.16)	Reference	-0.006 (-0.07 to 0.05)	Reference
Baseline	34	0.04 (-0.06 to 0.14)	0.05	0.09 (-0.12 to 0.30)	0.35
During follow-up	481	0.17 (0.14 to 0.21)	0.003	-0.24 (-0.31 to -0.16)	<0.0001
Change in AHEI score					
Increase (≥1 SD)	717	0.11 (0.07 to 0.16)	0.74	-0.05 (-0.14 to 0.05)	0.58
Stable (-1≤SD<1)	3071	0.11 (0.07 to 0.15)	Reference	-0.06 (-0.15 to 0.03)	Reference
Decrease (<-1 SD)	681	0.12 (0.07 to 0.16)	0.67	-0.05 (-0.14 to 0.04)	0.72
Change in physical activity					
Increase (≥1 SD)	601	0.16 (0.11 to 0.20)	0.0003	-0.09 (-0.19 to 0.005)	0.19
Stable (-1≤SD<1)	3312	0.11 (0.07 to 0.15)	Reference	-0.06 (-0.15 to 0.03)	Reference
Decrease (<-1 SD)	556	0.08 (0.03 to 0.12)	0.006	-0.002 (-0.10 to 0.10)	0.03
Change in BMI					
Increase (≥1 SD)	566	-0.01 (-0.06 to 0.03)	<0.0001	0.27 (0.17 to 0.37)	<0.0001

Stable (-1≤SD<1)	3367	0.11 (0.07 to 0.15)	Reference	-0.07 (-0.15 to 0.02)	Reference
Decrease (<-1 SD)	536	0.25 (0.20 to 0.29)	<0.0001	-0.36 (-0.46 to -0.25)	<0.0001
Change in alcohol consumption					
Increase (≥1 SD)	485	0.20 (0.15 to 0.24)	<0.0001	-0.009 (-0.11 to 0.09)	0.10
Stable (-1≤SD<1)	3564	0.12 (0.08 to 0.16)	Reference	-0.05 (-0.14 to 0.04)	Reference
Decrease (<-1 SD)	420	0.03 (-0.02 to 0.74)	<0.0001	-0.10 (-0.20 to 0.002)	0.18
Smoking status at follow-up					
Never‡	3951	0.11 (0.07 to 0.15)	Reference	-0.03 (-0.11 to 0.05)	Reference
Ex-smoker#	208	0.15 (0.10 to 0.20)	0.02	-0.08 (-0.20 to 0.03)	0.21
Current	310	0.08 (0.04 to 0.13)	0.18	-0.04 (-0.15 to 0.06)	0.75

HDL = high-density lipoprotein; CI = confidence interval; SD = standard deviation.

* After adjustment for sex, age, ethnicity, educational level, BMI, and longstanding illness at baseline, and duration of follow-up. Predictors were mutually adjusted.

† P-value for relative difference.

‡ Non-smoker at baseline and at follow-up.

Stopped smoking after baseline (phase 3).

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eTable 7. Proportion of variance (R^2) explained by lipid lowering drug, and change in diet and physical activity (n=4,469).

Models	R^2 (%)	ΔR^2 (%)
*Baseline**	*11.6*	*Ref*
Baseline + start of lipid lowering drug	41.0	29.4
Baseline + change in AHEI score	12.1	0.5
Baseline + change in physical activity	11.9	0.3
Baseline + start of lipid lowering drug	*41.0*	*Ref*
Baseline + start of lipid lowering drug + change in AHEI score	41.3	0.3
Baseline + start of lipid lowering drug + change in physical activity	41.3	0.3
Baseline + change in AHEI score	*12.1*	*Ref*
Baseline + change in AHEI score + start of lipid lowering drug	41.3	29.2
Baseline + change in AHEI score + change in physical activity	12.3	0.2
Baseline + change in physical activity	*11.9*	*Ref*
Baseline + change in physical activity + start of lipid lowering drug	41.3	29.4
Baseline + change in physical activity + change in AHEI score	12.3	0.4

* Baseline model includes sex, age at baseline, ethnicity, duration of follow-up, education level, BMI at baseline, and having a longstanding illness at baseline. $\Delta R^2 = R^2$ from the current model - R^2 from the reference model.

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