Ideal cardiovascular health influences cardiovascular disease risk associated with high lipoprotein(a) levels and genotype: The EPIC-Norfolk prospective population study

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Abstract
Background and aims: Lipoprotein(a) (Lp(a)) is a strong genetic risk factor for cardiovascular disease (CVD). The American Heart Association has prioritised seven cardiovascular health metrics to reduce the burden of CVD: body mass index, healthy diet, physical activity, smoking status, blood pressure, diabetes and cholesterol levels (together also known as ideal cardiovascular health). Our objective was to determine if individuals with high Lp(a) levels could derive cardiovascular benefits if characterized by ideal cardiovascular health.

Methods: A total of 14,051 participants of the EPIC-Norfolk study were stratified according to the cardiovascular health score (based on the number of health metrics with an ideal, intermediate or poor status). Of them, 1732 had a CVD event during a mean follow-up of 11.5 years. Cox proportional hazards models were used to describe the association between the cardiovascular health score and Lp(a) level or genotype (as estimated by the rs10455872 variant) with the risk of CVD.

Results: We observed little or no differences in serum Lp(a) levels across the seven cardiovascular health metric categories. Among participants with high serum Lp(a) levels (>50 mg/dL), those in the highest (i.e. healthiest) cardiovascular health score category (10–14) had an adjusted hazard ratio for cardiovascular disease of 0.33 (95% CI = 0.17 – 0.63, p = 0.001) compared to participants in the lowest (i.e. unhealthiest) cardiovascular health score category (0–4). Similar results were obtained when we replaced Lp(a) with rs10455872.

Conclusions: Although Lp(a) levels are only slightly influenced by cardiovascular health metrics, an ideal cardiovascular health could substantially reduce CVD risk associated with high Lp(a) levels or genotype.

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1. Introduction

Lipoprotein(a) [Lp(a)] consists of a cholesterol rich lipoprotein particle analogous to low-density lipoprotein (LDL), where apolipoprotein B-100 (the most abundant protein on LDL particles) is linked to apolipoprotein (apo)(a) by a disulphide bond. Individuals with Lp(a) levels in the top 20% of the population distribution (i.e. above approximately 50 mg/dL) have a 2–3 fold higher increased risk of coronary heart disease and stroke [1,2]. In fact, recent studies suggest that Lp(a) could be one of the strongest genetic risk factor for cardiovascular disease (CVD) [3]. The LPA gene is highly polymorphic and one of the strongest determinants of Lp(a) levels is a copy number variation (CNV) at the LPA locus encoding the number of KIV-2 repeats [4]. The single nucleotide
polymorphism (SNP) rs10455872 has been shown to be closely
associated with KIV-2 repeats and Lp(a) levels [5,6]. These genetic
variations at the LPA locus have been suggested to strongly influ-
ence Lp(a) levels by altering its hepatic secretion rate [4]. Up to 90% of
the variance in circulating Lp(a) could be explained by genetic
factors [7]. Lp(a) is the preferential carrier of oxidized phospho-
lipids in the blood [8], which may be the underlying reason for its
atherogenic potential.

In 2010, a European Atherosclerosis Society Consensus Panel
recommended that Lp(a) levels should be measured in individuals
with premature CVD (or with a family history of premature CVD),
familial hypercholesterolemia, recurrent CVD despite statin treat-
ment or intermediate cardiovascular risk [1]. However, even in these
populations, Lp(a) is currently not routinely measured. The
lack of a consensus on a validated and standardized Lp(a) assay, our
incomplete understanding of the atherogenicity of Lp(a) and
relative unawareness of Lp(a) amongst health care providers
probably are factors that could explain why Lp(a) is not routinely
measured. Moreover, a clinically validated specific therapy against
Lp(a) is not available.

The American Heart Association (AHA) 2020 Strategic Impact
Goals introduced the concept of ideal cardiovascular health, which
includes seven cardiovascular health metrics, namely body mass
index, a healthy diet, physical activity, smoking status, blood
pressure, fasting plasma glucose and cholesterol levels [9]. Over
the past 5 years, several prospective studies have shown that compared
to people who meet few of the criteria of ideal cardiovascular
health, those with ideal cardiovascular health have a 80–90% lower
risk of CVD events [10–12]. Whether individuals with high Lp(a)
can reduce their risk of cardiovascular events by controlling other
risk factors such as those of the AHA’s ideal cardiovascular health
is unknown. In this study, we hypothesised that Lp(a) levels will be
comparable across the seven healthy lifestyle risk factors and that
individuals with high Lp(a) levels (or carriers of G allele of the
Lp(a)-raising SNP rs10555872) are at lower risk of cardiovascular
events if they are characterized by ideal cardiovascular health. We
tested these hypotheses in the European Prospective Investigation
into Cancer and Nutrition (EPIC)-Norfolk study.

2. Materials and methods

2.1. Study design

The EPIC-Norfolk prospective population study is a population-
based cohort of 25,639 men and women, aged between 39 and 79
years, resident in Norfolk, United Kingdom. The design and
methods of the study have been described previously [13]. Partici-
pants were recruited from age-sex registers of general practices in
Norfolk as part of the 10-country collaborative EPIC study. The
study cohort was closely similar to UK population samples for many
characteristics, including anthropometry, blood pressure and lipids,
but with a lower proportion of smokers. At the baseline survey
conducted between 1993 and 1997, participants completed a
detailed health and lifestyle questionnaire. Blood was taken by
venipuncture into plain and citrate tubes. Blood samples were
processed for various assays at the Department of Clinical
Biochemistry, University of Cambridge, or stored at −80 °C.

Participants were identified as having been hospitalised or
having died because of a cardiovascular event if the corresponding
International Classification of Disease (ICD)-10 code was recorded
as the underlying cause of hospitalisation or mortality. Hospitalised
participants were identified using their unique National Health
Service number linked with the East Norfolk Health Authority
(ENCORE) database. The ENCORE database identified all hospital
contacts throughout England and Wales for residents of Norfolk.
Death certificates were coded by trained nosologists according to
ICD-10. Deaths or hospitalisations were attributed to coronary
heart disease (CHD) if the underlying cause was coded by as ICD-10
codes 120–125, which encompass the clinical spectrum of CHD,
including unstable angina, stable angina and myocardial infarction.
Deaths or hospitalisations were attributed to stroke if the under-
lying cause was coded as ischaemic (I63, I65, I66) or haemorrhagic
stroke (I60–62). CVD was defined as either a CHD or stroke. The
follow-up was censored on 31 March 2008. The study protocol was
approved by the Norwich District Health Authority Ethics Com-
mittee and all participants gave written informed consent.

2.2. Definition of cardiovascular health metrics

Ideal cardiovascular health metrics were classified as ideal, in-
termediate or poor according the seven risk factors identified by
the AHA, as previously described [12]. Body mass index (BMI) was
classified as ideal if < 25 kg/m², as intermediate if 25–30 kg/m² or
as poor if ≥ 30 kg/m². A healthy diet score (HDS) was based on
intake of five dietary components. The first component was the
intake of sufficient amounts of fruit and vegetables; a consumption
of 4.5 or more cups per day was classified as meeting the guidelines.
Second, the weight of estimated daily fish consumption was
multiplied by seven and divided by 3.5 oz (portion size); if the value
was > 2, the participant was considered to consume two or more
servings per week. Third, for fibre-rich whole grains, participants
consuming three or more servings per day of 1 oz each were con-
sidered to meet the guidelines. The fourth and fifth HDS com-
ponents were low sodium intake (< 1500 mg per day was classified as
healthy) and low consumption of sugar-sweetened beverages
(<450 kcal per week was classified as healthy). The HDS was
calculated as the sum of the number of healthy food items, yielding
a HDS range of 0–5. HDS was categorised as ideal (>4), interme-
diate (2–3), or poor (<2). Physical activity was defined as ideal,
intermediate and poor if the status was active, moderately active or
moderately inactive, and inactive, respectively, as previously
described [14]. Smoking status was classified as ideal, intermediate
or poor if the study participant had never smoked, previously
smoked, or was a current smoker, respectively. Blood pressure was
defined as ideal if systolic pressure was < 120 mmHg and diastolic
pressure was < 80 mmHg, as intermediate if systolic pressure was
120–139 mmHg or diastolic pressure was 80–89 mmHg without
antihypertensive drug treatment or if the blood pressure was at
goal on treatment, or poor if systolic pressure was ≥ 140 mmHg
or diastolic pressure was ≥ 90 mmHg. Total cholesterol levels were
classified as ideal (< 5.2 mmol/L), intermediate (5.2–6.2 mmol/L)
or when cholesterol levels were treated to goal, or poor (> 6.2 mmol/
L). Diabetes mellitus status was ascertained by means of (1) self-
report of diabetes medication use or (2) a Hba1c ≥ 6.5 mmol/L.
Participants meeting one of these criteria were attributed a value of
0 while individuals without diabetes mellitus were attributed a
value of 2.

The overall cardiovascular health score (CHS) was calculated
based on these seven health metrics, giving 2 points for an ideal
metric, 1 point for an intermediate metric, and 0 points for a poor
metric, thus yielding an overall CHS between 0 and 14. The CHS was
divided into three categories as follows: 0–4 (unhealthy), 5–9
(intermediate) and 10–14 (healthy).

2.3. Genotyping and laboratory measurements

The rs10455872 genetic variant was genotyped using Custom
TaqMan® SNP Genotyping Assays (Applied Biosystems, Warrington,
UK). The genotyping assays were carried out using 10 ng of genomic
DNA in a 2.5 µl reaction volume on 384-well plates using a G-Storm
GS4 Thermal Cycler (GRI, Rayne, UK). The ABI PRISM® 7900HT Sequence Detection System (Applied Biosystems, Warrington, UK) was used for end-point detection and allele calling. The SNP passed the quality control criteria in the EPIC-Norfolk study (call rate > 95%, blind duplicate concordance ≥ 95%). Various laboratory measurements including a conventional lipid profile, were performed at baseline as previously described [13]. When additional funding became available in 2010, additional measurements were performed in a subset of the cohort with available stored frozen blood samples. Lp(a) levels were measured with an immuno-turbidimetric assay using polyclonal antibodies directed against epitopes in apolipoprotein(a) (Denka Seiken, Coventry, United Kingdom), as previously described [15]. This assay has been shown to be apolipoprotein(a) isoform-independent.

2.4. Statistical analyses

Baseline characteristics of study participants were compared between participants with high vs. low Lp(a) levels using an unpaired Student t-test for continuous variables with a normal distribution or a chi-square test for categorical variables. Differences in median Lp(a) levels across cardiovascular health metrics with the categories of diabetes mellitus were tested using Kruskal–Wallis one-way analysis. For the difference in median Lp(a) levels across the diabetes mellitus group, a Mann–Whitney U test was performed. The differences in percentages of participants with high Lp(a) levels (> 50 mg/dL) and rs10455872 carriers across categories of ideal cardiovascular health metrics was tested using chi-square analysis. Cox proportional hazards models were used to calculate hazard ratios (HR) and corresponding 95% confidence interval (95% CI) for the risk of future CHD in participants separated on the basis of cardiovascular health metrics and Lp(a) levels (or genotype). Regression models were tested before and after adjustment for potential confounding risk factors such as age and sex. The interaction terms between Lp(a) and cardiovascular health and rs10455872 and cardiovascular health were calculated with the use of Cox proportional hazard models. Statistical analyses were performed using SPSS software version 20.

3. Results

A total of 14,051 participants with a complete data set available for ideal cardiovascular health metrics and Lp(a) levels were included in this analysis. Of them, 1732 had a CVD event during a mean follow-up of 11.5 years. The baseline characteristics of the study participants are presented in Table 1 for the entire study sample separated on the basis of Lp(a) levels (< 50 or ≥ 50 mg/dL). There were no sex and age differences between participants with high vs. low Lp(a) levels. Body mass index, blood pressure, the percentage of participants with diabetes mellitus or smokers was also comparable between participants with high vs. low Lp(a). Cholesterol levels were higher in participants with high Lp(a). With the exception of sodium intake, all other intake parameters were not different in patients with high vs. low Lp(a) for healthy diet components or physical activity habits.

Table 2 presents median Lp(a) levels in patients with ideal, intermediate or poor cardiovascular health metrics. Lp(a) levels were significantly different across diet and physical activity categories but these differences were very small and the percentage of participants with Lp(a) levels ≥ 50 mg/dL or carriers of the G allele of rs10455872 was not different across physical activity and diet categories. As anticipated, cholesterol levels were different across Lp(a) categories, most likely because Lp(a) carries cholesterol. Consequently, Lp(a) levels appeared to be slightly influenced by the presence/absence of the cardiovascular health score.

The associations between the cardiovascular health score, Lp(a) levels and cardiovascular outcomes are presented in Fig. 1. Fig. 1A shows that among patients with high Lp(a), those with ideal cardiovascular health have a relative risk of CVD of 0.33 (95% CI, 0.17–0.63, p < 0.001) compared to those with poor cardiovascular health. Our results also show that patients with ideal cardiovascular health and low Lp(a) levels are those with the lowest CVD event rate (hazard ratio = 0.19, 95% CI, 0.12–0.31, p < 0.001). Similar results were obtained when we replaced Lp(a) with the Lp(a)-raising allele (G allele of rs10455872). Fig. 1B shows that among study participants with at least one Lp(a)-raising allele, those with ideal cardiovascular health have a relative risk of CVD of 0.24 (95% CI, 0.13–0.42, p < 0.001) compared to those with poor cardiovascular health. Our results also show that patients with ideal cardiovascular health who did not have the Lp(a)-raising allele are those with the lowest CVD event rate (hazard ratio = 0.19, 95% CI, 0.13–0.28, p < 0.001). We also computed the interaction terms between Lp(a) levels (or genotype) and health categories for CVD risk prediction and found that none were significant (data not shown), which suggests that there is no evidence that the relationship between health categories and CVD risk is affected by Lp(a) levels (or genotype).
4. Discussion

Family-based studies and genetic association studies conducted in the general population have consistently shown that the inter-individual variation in Lp(a) levels are most likely explained by genetic factors. In this study, we found that the seven AHA metrics of ideal cardiovascular health merely influence Lp(a) levels, which further supports the notion that lifestyle may not represent a key a factor in the management of high Lp(a) levels. However, the results of our study do suggest that among people with high Lp(a) levels, the management of lifestyle-related factors may reduce cardiovascular risk by up to 75%.

Cross-sectional and intervention studies have been performed to address the impact of physical activity levels and exercise training on Lp(a) levels. Mora et al. found no association between physical activity levels and Lp(a) levels in the Women’s Health Study [16]. This is in line with the outcomes of an intervention study where nine-months of aerobic training in 30 healthy adults was shown not to have an impact on Lp(a) levels. In patients with type 2 diabetes, resistance training resulted in a decrease in Lp(a) levels (from 15.4 ± 18 mg/dL to 13.8 ± 18 mg/dL, p = 0.04) [17], but another study found no such significant effect [18]. In our study, a healthy dietary pattern was not strongly associated with Lp(a) levels. Dietary intervention studies have, however, suggested that the macronutrient composition of the diet may influence Lp(a) levels. For instance, in a randomized crossover study, Faghihnia et al. [19] have shown that a 4-week low-fat, high-carbohydrate diet increased Lp(a) levels compared to a high-fat, low-carbohydrate diet in 63 healthy participants. In the Omni Heart trial, Haring et al. [20] compared the impact of 3 diets (high-carbohydrate, high-protein and high in unsaturated fats) on Lp(a) levels. Although changes in Lp(a) were modest (2.1 to 4.7 mg/dL), all diets significantly increased Lp(a) levels, with marked heterogeneity among black and white participants. Investigators who have measured Lp(a) levels in nutritional intervention studies aiming at reducing LDL cholesterol levels (with portfolio diet, almonds, flaxseed, etc.) also found little benefits of these interventions with regards to Lp(a) levels [21–23]. Drugs targeting LDL cholesterol levels such as statins also have little or no effect on Lp(a) levels. In fact, whereas most studies showed that statins do not reduce Lp(a) levels, some studies show that statins could even increase Lp(a) and oxPL levels [24–26]. Whether people with high Lp(a) levels would derive more benefits from statin therapy than people with lower Lp(a) levels is unknown.

Our study has limitations. For instance, our study population almost exclusively included Caucasians. Therefore, our results may not be applicable to other populations. Although we provide results from a large-scale prospective study with a follow-up of 11.5 years, the true impact of encouraging people (with or without elevated Lp(a) levels) to improve cardiovascular health by meeting the AHA criteria on CVD outcomes can only be formally tested in a randomized clinical trial. However, regardless of Lp(a) levels, there are currently no randomized clinical trials that have documented the potential benefits of meeting the AHA’s criteria for ideal cardiovascular health.

The association between Lp(a) levels and CVD risk is strong and consistent across sexes and ethnicities. The measurement of Lp(a) is clinically useful as it enhances cardiovascular risk reclassification and discrimination, as recently observed in the Bruneck study and the Copenhagen City Heart Studies [27,28]. To the best of our knowledge, our study is the first to document the benefits of ideal cardiovascular health in patients with high Lp(a). Previous analyses within the EPIC-Norfolk dataset have confirmed that high Lp(a) levels are significantly associated with future risk of peripheral arterial disease, coronary artery disease and aortic stenosis [5,15], a finding that underscores the need for compounds that specifically target Lp(a) [reviewed in Ref. [29]] as they could provide substantial cardiovascular benefits to patients with high Lp(a) if properly tested in cardiovascular outcomes trials. However, our results suggest that if Lp(a)-lowering therapies should become available, these should be added on top of lifestyle management and on top of other agents that target risk factors for CVD such as LDL cholesterol.
and blood pressure in people who cannot manage these risk factors with lifestyle alone.

It is commonly accepted that one of the barriers to the routine measurement of Lp(a) levels in clinics is the absence of Lp(a)-lowering therapy. Although randomized clinical trials will ultimately be required to formally test this hypothesis, our results suggest that the management of seven other CVD risk factors could reduce CVD risk in patients with high Lp(a). We believe that our results should encourage health professionals to measure Lp(a) levels at least once in their patients and more routinely in patients treated with lipid-lowering therapy as well as other lifestyle-related risk factors to properly assess cardiovascular risk. In conclusion, our results suggest that controlling cardiovascular risk factors and prescribing physical activity and a healthy diet should be pivotal for the management of patients with high Lp(a).

**Conflict of interest**

The authors declared they do not have anything to disclose regarding conflict of interest with respect to this manuscript.

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**Author contributions**

NP designed the statistical analysis plan, interpreted the data and wrote the manuscript. RV performed statistical analyses, interpreted the data and reviewed the manuscript. MS coordinated the genotyping and reviewed the manuscript. SMB performed statistical analyses, interpreted the data and reviewed the manuscript. GKH interpreted the data and reviewed the manuscript. NJW coordinated the study and reviewed the manuscript. KTK coordinated the study and reviewed the manuscript. BJA designed the statistical analysis plan, interpreted the data and wrote the manuscript.

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