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ORIGINAL PAPER

Low serum lathosterol levels associate with fatal cardiovascular disease and excess all-cause mortality: a prospective cohort study

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Abstract

Importance A more precise identification of patients at "high cardiovascular risk" is preeminent in cardiovascular risk stratification.

Objective To investigate the relationships between markers of cholesterol homeostasis, cardiovascular events and all-cause mortality.

Design, setting and participants We quantified markers of cholesterol homeostasis by gas chromatography–mass spectrometry in 377 subjects with suspected coronary artery disease, who were not on lipid-lowering drugs at baseline. All patients were followed for occurrence of cardiovascular events and mortality over a period of 4.9 +/− 1.7 years. The standardized mortality ratio (SMR) was calculated as the ratio of the observed and the expected deaths based on the death rates of the Regional Databases Germany, and Poisson regression (rate ratio, RR) was used to compare subgroups. The SMR and RR were standardized for sex, age category and calendar period. In addition, Cox regression (Hazard ratio, HR) was used to determine the effect of co-variables on (cardiovascular) mortality within the cohort.

Main outcomes Cardiovascular events, cardiovascular mortality and all-cause mortality.

Results A total of 42 deaths were observed in 1818 person-years corresponding with an SMR of 0.99 (95% CI 0.71–1.33; $p = 0.556$). A fatal cardiovascular event occurred in 26 patients. Lower levels of lathosterol were associated with increased cardiovascular mortality (HR 1.59; 95% CI: 1.16–2.17; $p = 0.004$) and excess all-cause mortality (HR 1.41; 95% CI: 1.09–1.85; $p = 0.011$). Lower lathosterol tertile compared to the adjacent higher tertile was associated with 1.6 times higher all-cause mortality risk (RR 1.60; 95% CI 1.07–2.40; p for trend = 0.022). This corresponded with a 2.3 times higher mortality risk of a lathosterol–LDL ratio equal to or below the median (RR 2.29; 95% CI 1.19–4.43; $p = 0.013$). None of the other cholesterol homeostasis markers were associated with cardiovascular and all-cause mortality.

Conclusions In patients not on lipid-lowering agents, low serum lathosterol correlated with increased risk of cardiovascular events and excess all-cause mortality.

Keywords Cholesterol · Synthesis · Absorption · Mortality · Cardiovascular events

Introduction

An elevated plasma concentration of low-density lipoprotein cholesterol (LDL-C) is a primary causal factor in the development of atherosclerotic cardiovascular vascular disease [1]. Large-scale primary and secondary prevention trials with statins have demonstrated a marked reduction in cardiovascular events and mortality [2]. Serum cholesterol levels are regulated by both cholesterol synthesis and cholesterol absorption [3]. Lathosterol, a precursor in cholesterol synthesis, is a marker of the endogenous cholesterol synthesis rate, and plant sterols such as campesterol and sitosterol reflect the efficacy of the individual cholesterol absorption capacity. Data from a subgroup analysis of the 4-S-Study indicated as early as 1998 that patients with high cholesterol absorption and low cholesterol synthesis may

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not benefit from statin treatment [4]. In patients with severe aortic stenosis, who were not treated with lipid-lowering drugs, we demonstrated that low cholesterol synthesis and high cholesterol absorption were significantly associated with coronary artery disease (CAD) [5]. Similar findings were reported from a case–control study nested into the Framingham Offspring Study [6]. Notably, in both studies, markers of cholesterol homeostasis were better predictors for CAD than traditional lipid risk factors. Similar findings were reported in the LURIC study, a prospective study evaluating patients admitted for coronary angiography in Germany [7]. Therefore, we have previously called for prospective studies of cholesterol homeostasis to assess cardiovascular risk stratification on an individual basis. In addition to the relationship with cardiovascular events and coronary morbidity, we investigated if markers of cholesterol homeostasis are associated with fatal cardiovascular events and all-cause mortality, the most indisputable endpoint. For this purpose, we have performed an analysis of markers of cholesterol homeostasis restricted to the subgroup of patients, who were not receiving lipid-lowering agents at baseline, of the HOM SWEET HOMe study, which is a prospective cohort study in patients with suspected coronary heart disease [8].

Methods

Plasma samples were taken and frozen from patients who underwent coronary angiography in the University Hospital of the Saarland [8]. We excluded subjects taking lipid-lowering medication (statins, ezetimibe, fibrates) at baseline, as cholesterol-lowering drugs have a strong impact on lathosterol levels [9]. A total of 377 untreated subjects were included in the present study. Cholesterol was quantified after gas chromatographic (GC) separation and flame ionization detection using 5α-cholestane as internal standard [10, 11]. Campesterol, sitosterol and lathosterol were determined by GC–mass selective detection (MSD) using epicoprostanol as internal standard [10]. Demographic data and continuous variables were summarized as mean ± SD or median (25%–75% CI).

The standardized mortality ratio (SMR) was calculated as the ratio of the observed and the expected deaths. The latter were calculated using the population death rates obtained from the Regional Database Germany (weighted on the inclusion from Rheinland-Pfalz and Saarland). To compare all-cause mortality between subgroups, Poisson regression (rate ratio, RR) was used. The SMR and RR were standardized for sex, age category and calendar period.

In addition, Cox regression (Hazard ratio, HR) was used to determine the effect of co-variables on cardiovascular and all-cause mortality within the cohort adjusted for gender and age using STATA (version 15.0. STATA Corp, College Station, TX). We considered age, sex, body mass index, glomerular filtration rate, systolic blood pressure, total cholesterol, low-density lipoprotein, high-density lipoprotein, triglycerides, smoking, antihypertensive medication, and diabetes as candidate explanatory covariates and performed backward variable selection in a Cox proportional hazards model for all-cause mortality using a significant level of greater than $p = 0.05$ for removal from the model. Moreover, we applied the 10% change-in-estimate method of variable selection. All cholesterol metabolism markers were log-transformed to correct for their skewed distributions and standardized to be comparable. Values indicate HR per 1 SD of the natural logarithm with 95% CIs. p values below 0.05 were considered as statistically significant.

Results

The average age of subjects included in the analysis was 64.6 years, 63.3% were males. A median body mass index of 27.7 (24.7–30.9 CI) kg/m^2 indicates a tendency towards overweight. Current smoking was reported by 19.7%; 19.4% reported a family history of cardiovascular diseases; and 31.4% had diabetes (Table 1). This cohort is representative for patients undergoing cardiac catheterization in Germany.

In 1818 person-years, 44 patients suffered a cardiovascular event and 42 deaths were observed. The SMR was 0.99 (95% CI 0.71–1.33; $p = 0.556$). Out of 42 deaths, 26 were based on a cardiovascular event. A lower lathosterol tertile compared to the adjacent higher tertile was associated with 1.6 times higher all-cause mortality risk (RR 1.60; 95% CI

Table 1 Baseline patients characteristics

Total ($n = 377$)	
CV death	6.9% (26)
Male sex	63.3% (238)
Age (years)	64.6 ± 10.6
Body mass index (kg/m^2)	28.3 ± 4.9
Waist circumference (cm)	99.6 ± 13.8
Waist–hip ratio	1.0 ± 0.1
eGFR (mi/min/1.73 m^2)	81.1 ± 21.3
LVEF (%)	69.0 (59.0–75.0)
Systolic BP (mm Hg)	132.8 ± 21.5
Peripheral artery disease	10.9% (41)
Smoking	19.7% (74)
History of CAD	17.8% (67)
History of stroke	6.4% (24)
Diabetes	31.4% (118)
Positive family history	19.4% (73)
HbA1c (%)	5.7 (5.4–6.4)
C-reactive protein (mg/l)	2.3 (1.1–4.5)

1.07–2.40; p for trend = 0.022). This corresponded with a 2.3 times higher mortality risk of a lathosterol–LDL ratio equal to or below the median (RR 2.29; 95% CI 1.19–4.43; $p = 0.013$). None of the other cholesterol homeostasis markers were associated with cardiovascular and all-cause mortality. The database was virtually complete: all variables were available for all cases except for one man, whose measurements of total cholesterol, triglycerides, LDL cholesterol and HDL cholesterol were missing and one man, whose LDL cholesterol was missing. These two persons, whose lipid profiles were incomplete, were alive at sensoring. The two men contributed 12.8 person-years to the total follow-up corresponding with a negligible expected death rate. In the remaining 375 patients, LDL cholesterol levels below the median value (3.549 mmol/l) were not associated with a significantly improved life expectancy (RR 0.94; 95% CI 0.51–1.73; $p = 0.851$). In line, adjustment for the individual LDL cholesterol levels did not influence our results.

Using Cox proportional hazards models adjusted for age and gender, lower levels of lathosterol, lathosterol-to-cholesterol and lathosterol-to-LDL were associated with a higher risk for cardiovascular mortality (Fig. 1). Moreover, patients in the lowest tertile of lathosterol demonstrated a higher incidence of CVEs than patients in tertiles 2 and 3 (Fig. 2).

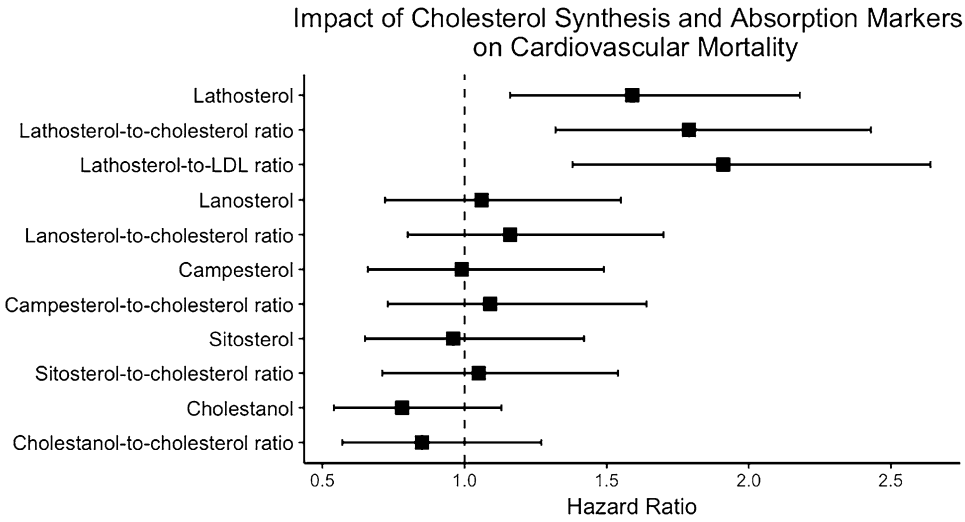

Fig. 1 Markers of cholesterol homeostasis and cardiovascular mortality. Forest plot. Lower levels of lathosterol, lathosterol-to-cholesterol and lathosterol–LDL ratio are associated with an increased risk for cardiovascular mortality

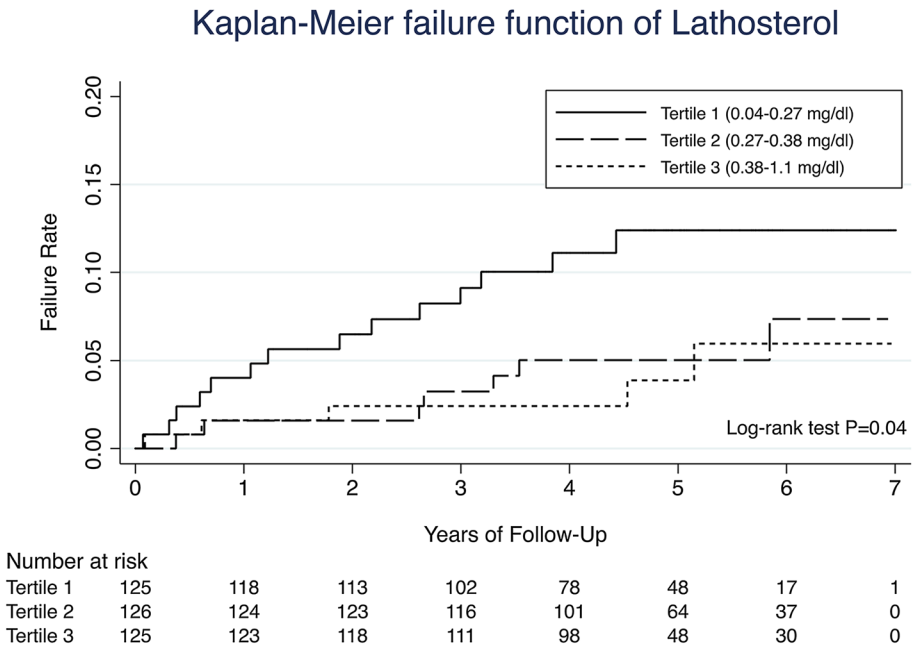

Fig. 2 Kaplan–Meier failure function for lathosterol tertile

Discussion

We confirmed that low serum levels of lathosterol, which is a marker for endogenous cholesterol synthesis, are associated with cardiovascular events and found excess mortality as well. Our observations were made within the cohort as well as in a nested case comparison with the general population of south-west Germany.

We believe that these findings are of importance for a number of reasons: first, low lathosterol serum levels might serve as a marker to identify patients at excess cardiovascular risk. This requires confirmation in large population-based prediction studies. Identification of patients with low lathosterol levels may indicate a need for early start of treatment.

Second, it is tempting to speculate that increasing LDL receptors—a mechanism shared by statins and PCSK9 inhibitors—might be more important for cardiovascular risk reduction than inhibiting cholesterol synthesis [12]. In case of a naturally occurring low endogenous cholesterol synthesis, the increase of the expression of LDL receptors during statin treatment may be limited and—in line—this may result in a low efficacy.

Finally, findings in this study raise the question of how to treat patients with low serum levels of lathosterol. Since low endogenous cholesterol synthesis is associated with increased cholesterol absorption, it can be speculated that this patient subset benefits in particular from cholesterol absorption inhibitors such as Ezetimibe [13, 14]. This idea was recently supported by a subgroup analysis of the HIJ-PROPER Study [15]. Nonetheless, this hypothesis needs to be tested in dedicated prospective studies.

Our study has several limitations. Although the analysis was performed prospectively, the study was not primarily set up for this evaluation. Since lipid-lowering agents have a major impact on lathosterol serum levels, we had to exclude patients who were on any lipid-lowering agent at baseline and the treatment regimes during the follow-up period were not documented. However, the initially high-risk patients in our analysis obviously belong to a well-treated cohort, as the cardiovascular event rates were very low resulting in a life expectancy identical to the general population of the region.

In conclusion, untreated persons with low serum lathosterol had increased risk of cardiovascular events and excess all-cause mortality. Markers of cholesterol homeostasis, in particular lathosterol, may improve individual cardiovascular risk prediction and might guide treatment options prior to starting lipid-lowering therapies. However, further prospective and interventional studies are required to confirm these findings.

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