Elevated HDL cholesterol levels: always beneficial?

Luisa Zanolla[1], Maria Stella Graziani[2], Giuseppe Lippi[3]
[1]Università degli Studi di Verona, Sezione di Cardiologia
[2]Università degli Studi di Verona, Sezione di Biochimica Clinica
[3]Università degli Studi di Verona, Sezione di Biochimica Clinica e Scuola di Medicina

ABSTRACT

An impressive number of studies published during the past years have demonstrated that low levels of high-density lipoprotein cholesterol (HDL-C) are associated with increased cardiovascular risk. Nevertheless, more recent data in both ischemic heart disease patients and general population revealed that also considerably increased HDL-C concentration could be associated with enhanced risk of all-cause and cardiovascular mortality, displaying an U-shaped association with adverse outcomes. These paradoxical findings prompted us to review the available information on this matter, concluding that the association between extremely elevated HDL-C and mortality may not be totally unexpected or unpredictable. Some unfavorable consequences of high HDL-C levels are confirmed by epidemiologic data in over-trained subjects, in whom a similar U-shaped curve is also observed between strenuous training and mortality. This review is hence aimed to summarize these evidences, proffering some possible mechanisms underlying this enigmatic association. If this association could be confirmed in further studies, it may be advisable that laboratory reporting of HDL-C should encompass a range of "desirable values" rather than indicating a single lower decision threshold, as has been suggested until presently.

Kay-words: *HDL-cholesterol, mortality, cardiovasculare risk factor*

INTRODUCTION

At the end of May 2022, JAMA Cardiology published an epidemiological study (1) analyzing the association between high levels of HDL cholesterol (HDL-C) and mortality, in patients with known coronary artery disease (CAD). The study proved that very high levels of HDL-C were associated with increased risk of both cardiovascular and total mortality. This is an evidence that subverts the current knowledge, that low levels of HDL-C represent a risk factor in primary prevention (2), so that the use of the term "good cholesterol" has become widespread in the collective imagination. In secondary prevention, however, data availability is limited.

Many prospective observational studies, conducted worldwide, confirmed an inverse relationship between HDL-C and cardiovascular risk, independently of gender and ethnicity (3). Accordingly, it was assumed, by translation, that increasing HDL-C through lifestyle changes and/or pharmacological intervention could reduce the risk of cardiovascular disease.

Several lifestyle factors can influence HDL-C levels (4); it is well known, in fact, that there is an association between HDL-C levels and potentially harmful habits such as a sedentary lifestyle, smoking and obesity. There is thus a risk of interpreting prognostic variations as an effect of HDL-C variations, when in fact this could only represent a confounding variable[1].

Some *post-hoc* analyses of randomized controlled trials suggest that increasing HDL-C could positively modulate the risk of cardiovascular disease. In the Lipid Research Clinics Coronary Primary Prevention

[1] A confounding variable is a variable that correlates (positively or negatively) both with the exposure (the risk factor) and with the outcome. Confounding can be a major problem with any observational (non-randomized) study; ignoring confounding can often result in a biased or incorrect estimate of the association

Corresponding Author: Luisa Zanolla, Università degli Studi di Verona, Sezione di Cardiologia - Email: luisa.zanolla@univr.it

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Trial, which tested the effect of lipid lowering treatment with cholestyramine in middle-aged males with primary hypercholesterolaemia, over the course of the study each 1 mg/dL (0.026 mmol/L) increase in HDL-C was associated with a 4.4% reduction in the risk of death from CAD or myocardial infarction (5).

In the Helsinki Heart Study (6), testing the primary prevention effect of gemfibrozil in middle-aged men with primary dyslipidemia, a 1% increase in HDL-C was associated with a 3-4% reduction in cardiovascular mortality.

However, interventions specifically aimed at increasing the concentration of HDL-C in subjects treated with statins, did not evidence a significant reduction in cardiovascular events (7,8), or they even demonstrated an increase (9).

The purpose of this review is to analyze and comment the available evidence on the association of high HDL-C levels with mortality, to contribute to the dissemination of knowledge of this unexpected association, while providing some possible interpretations.

THE NEW EPIDEMIOLOGICAL EVIDENCE

The cited study (1) enrolled 14 408 patients with ischemic heart disease from two biobanks: the Emory Cardiovascular Biobank (EmCAB) (5 467 patients), which since 2003 has been enrolling patients older than 18 years undergoing coronary angiography, and the UK Biobank (UKB) (11 034 patients), which has been enrolling patients with known CAD since 2006. The median observation period was 8.4 years. Total mortality was identified as response variable, i.e., an endpoint with low risk of bias. Cardiovascular mortality was considered as a secondary response variable.

Five categories were defined according to baseline HDL-C level, with values <30, 30 to 40, 40 to 60, 60 to 80, and >80 mg/dL (<0.78; 0.78-1.03; 1.03-1.55; 1.55-2.07; >2.07 mmol/L) (1). The category between 40 and 60 mg/dL (1.03-1.55 mmol/L) was used as the reference. The statistical analysis was conducted separately for the two databases, because of differences in inclusion and evaluation criteria as well as in available parameters.
Survival analysis was adjusted for several covariates: age, gender, ethnicity (self-reported), body mass index, history of high blood pressure, diabetes, smoking history, triglyceride levels, LDL cholesterol levels, estimated glomerular filtration rate (eGFR) and frequent use of alcohol (defined for the UKB as drinking 3 or more times per week, and for the EmCAB as drinking 8 or more alcoholic beverages per week).

The association between HDL-C, as a continuous variable, and both total and cardiovascular mortality was evaluated with a non-linear statistical technique, with an HDL-C level of 55 mg/dL (1.42 mmol/L) as the reference value. The values suggested as "desirable" for HDL-C are approximately >40-45 mg/dL (1.03-1.16 mmol/L) for males and 45-50 mg/dL (1.16-1.30 mmol/L) for females (10).

In UK Biobank data, patients with HDL-C >80 mg/dL (2.07 mmol/L) were 1.8% of the population. They tended to be older, more often female, more frequently alcohol users, with lower prevalence of cardiovascular risk factors and lower triglyceride levels. Survival analysis was adjusted for the previously mentioned covariates and, in addition, for history of stroke and heart attack.

Patients with high HDL-C levels, >80 mg/dL (2.07 mmol/L), after adjustment for covariates, had higher total mortality with a hazard ratio (HR) of 1.96, 95% confidence interval (95%CI) 1.42–2.71; p<0.001 and cardiovascular mortality (HR 1.71; 95%CI 1.09–2.68; p=0.02) (Figure 1) compared to the reference category. Graph data are extrapolated from the original paper using WebPlotDigitizer (11). Even HDL-C values between 60 and 80 mg/dL (1.55-2.07 mmol/L) were associated with an increase in total mortality, compared to the reference category (HR 1.27; 95%CI 1.08-1.50; p=0.005). There was an interaction between total mortality and gender (p=0.04), reflecting a different trend between the two categories, with higher mortality in males (HR 2.63; 95%CI 1.75-3.95, p<0.01), while in women the increase did not reach the statistical significance (HR 1.39; 95%CI 0.82-2.35; p=0.23). Cardiovascular mortality had a borderline interaction with gender, which was not statistically

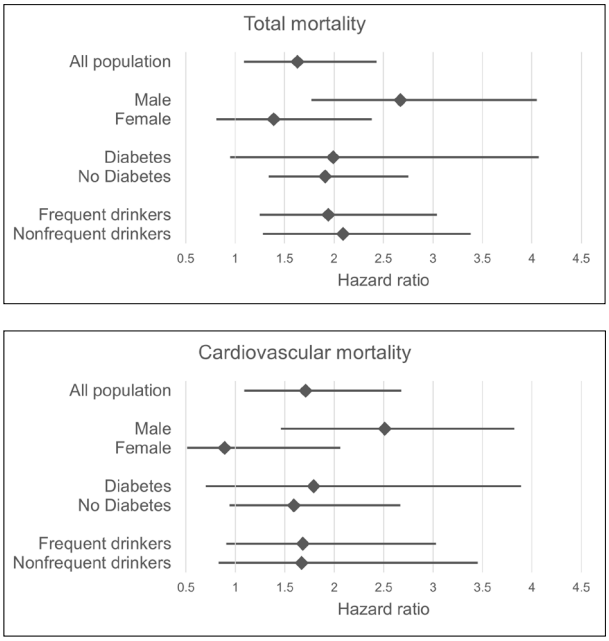

Figure 1
UK Biobank. Total and and cardiovascular mortality in patients with ischemic heart disease and HDL-cholesterol values ≥80 mg/dL (2.07 mmol/L). Data adjusted for age, gender, ethnicity, body mass index, history of high blood pressure, diabetes, smoking history, triglyceride and LDL cholesterol levels, history of stroke, history of heart attack, eGFR, and frequent use of alcohol, excluding the stratification variable. Both total and cardiovascular mortality increased compared to the baseline category of 55 mg/dL (1.42 mmol/L). The interaction between total mortality and gender is significant for total mortality, while that for cardiovascular mortality does not reach statistical significance. Total mortality was significantly increased in males, but not in females. No interaction was observed between mortality and alcohol consumption; the values are elevated in both habitual and non-habitual drinkers. From (1), modified.

significant (p=0.06) (Figure 1). Alcohol consumption is often regarded as a cause of HDL-C elevation , and indeed the proportion of frequent drinkers (n=5 571) increases as HDL-C levels rise. In the population with values >80 mg/dL (2.07 mmol/L), however, mortality values are significantly increased both in habitual drinkers (HR 1.93; 95%CI 1.25-2.99; p=0.003) and in non-habitual drinkers (HR 2.08; 95%CI 1.29-3.37; p=0.003) (Figure 1).

A genetic risk score (GRS) was calculated in the UK Biobank database, based on the 142 independent single nucleotide variations associated with HDL-C levels in a large genome wide association study (12). Adding the GRS to the statistical model did not modify the association between mortality and high HDL-C values. The genetic variations, thus quantified, do not appear to significantly contribute to the risk definition.

In the EmCAB data, patients with HDL-C >80 mg/dL (2.07 mmol/L) were 1.6%, more often black and female, with more frequent alcohol consumption, lower triglycerides value, lower prevalence of myocardial infarction, and less frequent use of statins, acetylsalicylic acid, β blockers, and renin-angiotensin system inhibitors. The survival analysis was adjusted for the previously mentioned covariates and, in addition, for history of heart failure and myocardial infarction. Compared to the reference category, between 40 and 60 mg/dL (1.03-1.55 mmol/L), patients with high levels of HDL-C, after adjustment for the covariates, had higher values of total mortality (HR 1.63; 95%CI 1.09-2.43; p=0.02) and a borderline increase in cardiovascular mortality (HR 1.57; 95%CI 0.95-2.61; p=0.08). In patients with high HDL-C levels, there was a significant interaction between cardiovascular mortality and age (p=0.01), with patients <65 years of age having a higher cardiovascular mortality (HR 3.65; 95%CI 1,52-6.11; p=0.002) than the older ones (HR 1.02; 95%CI 0.50-2.11; p=0.95) (Figure 2). In subjects with the highest HDL-C values, there was also an interaction between diabetes and mortality, both total and cardiovascular (p=0.03 and p=0.04, respectively), with increased risk in diabetics of both total (HR 3.03; 95%CI 1.62-5.65; p<0.001) and cardiovascular mortality (HR 3.14; 95% CI 1.55-6.38; p=0.002).

In both databases, the increased risk of death, both total and cardiovascular, appears independent of the classic cardiovascular risk factors, alcohol consumption, influenced only by male gender in the UK Biobank case series and by diabetes in the EmCAB case series.

Previous studies indicated that excessive alcohol intake may be a confounding factor, increasing the level of HDL-C (13); however, in this study, adjustment for frequency of alcohol intake did not attenuate the strong association between mortality and HDL-C levels in both populations.

The paper of Liu et al. (1) on the effect of high HDL-C levels in patients with ischemic heart disease attracted so much attention, that the journal dedicated an editorial (14). The two JAMA Editors point out that potential explanations for this U-shaped mortality increase with respect to HDL-C are likely multifactorial and may, at least in part, be attributed to biological effects of elevated HDL-C levels.

In the same year, the same Authors published a study, also based on the UK Biobank database, evaluating subjects with no known history of ischemic heart disease (15). The classification of the 451416 subjects enrolled, according to the HDL-C values, was carried out with the same categories of the previously mentioned study (1).
Overall, 6.9% of subjects had elevated HDL-C values; they had a trend to be older, more often female, with lower body mass index, lower triglycerides, lower rates of diabetes, hypertension, stroke, and heart attack, higher total cholesterol, and higher alcohol consumption. Statistical analysis was adjusted for age, gender, ethnicity, body mass index, hypertension, smoking, triglycerides, LDL cholesterol, history of stroke, history of heart attack, diabetes, eGFR, and alcohol use.

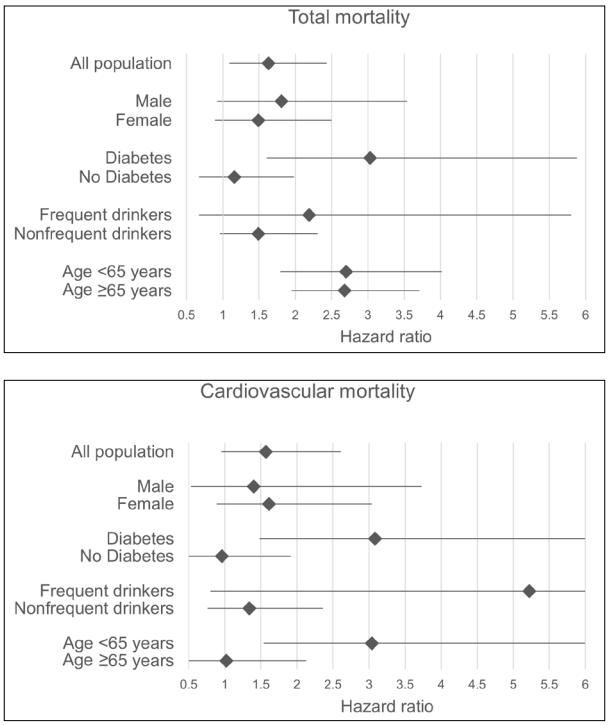

Figure 2

Emory Cardiovascular Biobank (EmCAB). Total and cardiovascular mortality in patients with ischemic heart disease and HDL-cholesterol values ≥80 mg/dL (2.07 mmol/L), compared to the baseline category, 40-60 mg/dL (1.03-1.55 mmol/L).
Data adjusted for age, gender, ethnicity, body mass index, history of hypertension, diabetes, smoking history, triglyceride and LDL cholesterol levels, history of heart failure, history of myocardial infarction, eGFR, frequent alcohol user, statin, aspirin, beta-blocker and renin-angiotensin system inhibitors use, excluding the stratification variable. In the analysis considering total mortality as a continuous variable, this is significantly increased compared to the reference category, (55 mg/dL; 1.42 mmol/L), while cardiovascular mortality shows a borderline statistical significant increase.
The interaction between cardiovascular mortality and age is statistically significant, with higher mortality in patients up to 65 years of age, compared to the older ones.
In the category with the highest HDL-C values, an interaction is also observed between the presence of diabetes and both total and cardiovascular mortality, with an increased risk of total and cardiovascular mortality in diabetics.
From (1), modified.

The category with HDL-C between 40 and 60 mg/dL (1.03-1.55 mmol/L) was used as a reference category also in this study. The association between HDL-C (as a continuous variable) and mortality (both total and cardiovascular) was evaluated with a non-linear statistical technique also in this paper, with an HDL-C level of 55 mg/dL (1.42 mmol/L) as a reference value. In subjects with HDL-C >80 mg/dL (2.07 mmol/L), over a 9 year observation period, there was an increase both of total (HR 1.11; 95%CI 1.03-1.20; p=0.005) and of cardiovascular mortality (HR 1.24; 95%CI 1.05-1.46; p=0.01).

There was a statistically significant interaction between gender and both total (p<0.0001) and cardiovascular (p<0.0001) mortality. The analysis according to the gender showed that high HDL-C values in women are not significantly associated with total (HR 0.97; 95%CI 0.88-1.06; p=0.5) (Figure 3 – left panel) or cardiovascular (Figure 3 – right panel) (HR 1.04; 95%CI 0.83-1.31; p=0.8) mortality, while in male subjects these values are associated with a significant increase in total (HR 1.79; 95%CI 1.59-2.02; p<0.0001) and cardiovascular (HR 1.92; 95%CI 1.52-2.42; p<0.0001) mortality. There was a significant interaction between HDL-C >80 mg/dL (2.07 mmol/L) and mortality as a function of frequency of alcohol consumption (p=0.03). However, when adjusted for gender, there was no interaction, but in men, the risk of mortality with HDL-C >80 mg/dL (2.07 mmol/L) was increased, independently from alcohol intake.

GRS was significantly associated with HDL-C levels (p<0.0001), but its inclusion into the statistical model left almost unchanged the association between mortality and elevated HDL-C values.

Male subjects, with no history of heart disease and very high HDL-C concentrations (>80 mg/dL; 2.07 mmol/L), being 2% of the entire male population, have nearly 2 times higher risk both of total and cardiovascular mortality. Female subjects, with no known history of heart disease and very high HDL-C concentrations (>80 mg/dL; 2.07 mmol/L), being 11% of the female population, did not show any difference, compared to the reference category, both for total mortality and for cardiovascular mortality.

OTHER EPIDEMIOLOGICAL EVIDENCE

Although only the publication of these two recent studies (1,15) raised a considerable interest with a significant resonance, an analysis of the literature prior to the two cited papers allows us to underpin how the relationship between high levels of HDL-C and mortality, at least in primary prevention, has already been described several times, albeit in less popular and prestigious scientific journals than those where the two most recent papers were published (1,15).

A meta-analysis published in 2020 (16), included 37 studies published between 1990 and 2019 and totaling 3 524 505 subjects from the general population, excluding those at high cardiovascular risk. Total mortality was non-linearly associated with HDL-C level, with a J shaped pattern, with minimum mortality at 54-58 mg/dL (1.40-1.50 mmol/L) (Figure 4 – upper panel). For values >58 mg/dL (1.50 mmol/L), total mortality increased by a HR of 1.03 (95%CI, 1.01, 1.05) for each 10 mg/dL (0.26 mmol/L) increase in HDL-C. This pattern of mortality was evident in both genders. Even cardiovascular mortality was related to HDL-C with a J-shape and minimum value for 68-71 mg/dL (1.76-1.84 mmol/L) (Figure 4 – lower panel). For higher values, cardiovascular mortality increased by a HR of 1.06 (95%CI, 1.01-1.10) for each 10 mg/dL (0.26 mmol/L) increase in HDL-C.In subjects with higher HDL-C values, cardiovascular mortality was increased compared to the reference category, i.e., the minimum value (HR 1.21; 95%CI 0.98 -1.49). The increase was even more evident in subjects on treatment with lipid-lowering drugs (HR 2.66; 95%CI 1.55-4.57).

An unusual result of this study is that mortality from ischemic heart disease had an inverse, non linear association with HDL-C levels, decreasing even for very high values. However, it should be noted that this result was based on only 7 studies, albeit with 1 634 events.

Among the mechanisms that support this epidemiological result, the Authors hypothesize a role for confounding factors, with particular attention to alcohol consumption. In the results of their meta-analysis, however, although studies reporting alcohol consumption had a higher likelihood of cardiovascular mortality (HR 1.32; 95%CI, 1.05-1.68) than those without alcohol consumption (HR 0.88; 95%CI 0.66-1.17), there was no significant interaction (p=0.16), so the data do not support this possible interpretation.

After the papers of the period covered by the aforementioned meta-analysis (16), a study was published (17) on a series of 42 145 patients enrolled in the National Health and Nutrition Examination Surveys (NHANES) 1999-2014. Unlike the study by Liu et al. (15), subjects at cardiovascular risk or with concomitant heart disease were not excluded from this study.

Results were adjusted for age, gender, ethnicity, education, smoking, body mass index, systolic blood pressure, eGFR, caloric intake, total cholesterol, comorbidities (hypertension, diabetes, cardiovascular disease, cancer), and drug therapy (antihypertensive and hypoglycemic medications, lipid-lowering drugs, antiplatelet agents).

Total mortality, compared to the reference group with HDL-C 51-60 mg/dL (1.32-1.55 mmol/L), displayed a J-shaped pattern even in this series (Figure 5, upper panel). A statistical analysis with a two-segment model showed that with respect to the threshold value of HDL-C 63 mg/dL (1.63 mmol/L), total mortality was increased for higher values with HR 1.51 (95%CI 1.30-1.76; p<0.01). Cardiovascular mortality had a nonlinear association with HDL-C (Figure 5, lower panel) in the two-segment model, relative to the cutoff of 46 mg/dL (1.19 mmol/L); for higher values, cardiovascular mortality showed a non statistically significant increase (HR1.11; 95%CI 0.89-1.40; p=0.36). After adjustment for covariates, even cardiovascular mortality reached the statistical significance (p=0.007).

In the subgroup analysis, adjusted for the covariates, a significant interaction emerged in the total mortality with

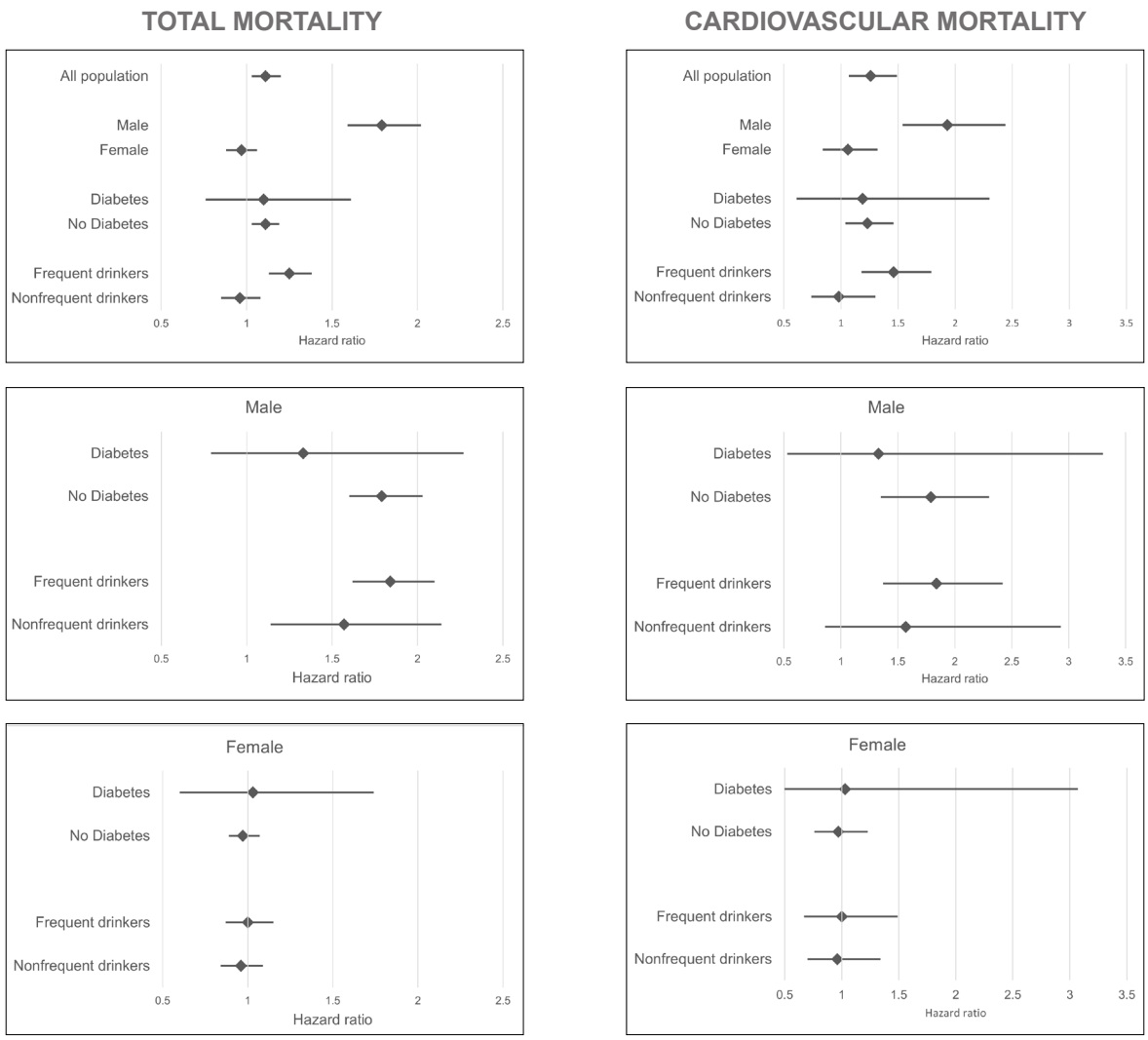

Figure 3

UK Biobank. Total (left panels) and cardiovascular mortality (right panels), in patients without ischemic heart disease and HDL-cholesterol values ≥80 mg/dL (2.07 mmol/L). Data adjusted for age, gender, ethnicity, body mass index, history of hypertension, diabetes, smoking history, triglyceride levels and LDL cholesterol levels, history of stroke, history of heart attack, eGFR, and frequent use of alcohol, excluding the stratification variable. Both total and cardiovascular mortality are significantly increased compared to the reference category, (40-60 mg/dL; 1.03-1.55 mmol/L). The interaction between both total and cardiovascular mortality and gender is significant. Total mortality is significantly increased in males, but not in females. The interaction between mortality and frequency of alcohol consumption is significant; however, when adjusted for sex, there is no interaction. In men, the risk of total mortality in the presence of elevated HDL cholesterol is increased, regardless of alcohol intake.
From (15), modified.

gender, ethnicity, obesity, and a borderline significance with arterial hypertension. The interaction with gender (p<0.01) evidenced that mortality was higher in women for low HDL-C values (Figure 6), and was higher in males for high HDL-C values, respectively. Trends according to ethnicity (p<0.001) were more difficult to interpret: there were higher mortality values for low HDL-C in both Caucasians (HR 1.43; 95%CI 1.16-1.76) and in non-Caucasians (HR 1.18; 95%CI 1.0-1.65); for HDL-C >70 mg/dL (1.81 mmol/L), the increased mortality was significant in non-Caucasians (HR 1.27; 95%CI 1.07-1.50), but failed to reach significance in Caucasians (HR 1.05 95%CI 0.91-1.21). Regarding obesity, for HDL-C >70 mg/dL (1.81 mmol/L) total mortality was significantly increased in non-obese (body mass index <25) with HR 1.18 (95%CI 1.00-1.39). For arterial hypertension, the interaction was borderline statistically significant (p=0.048), with significantly increased mortality values in hypertensives with HDL-C above >70 mg/dL 1.81 mmol/L) (HR 1 ,21; 95% CI 1.06-1.36).

Total mortality did not differ in the presence (n=3395) or absence (n=31868) of cardiovascular disease (p=0.33), nor for treatment (n=4895) or not (n=30368) with lipid-lowering drugs (p=0.80).

For cardiovascular mortality, only ethnicity had a significant interaction (p<0.01), but substantially for a

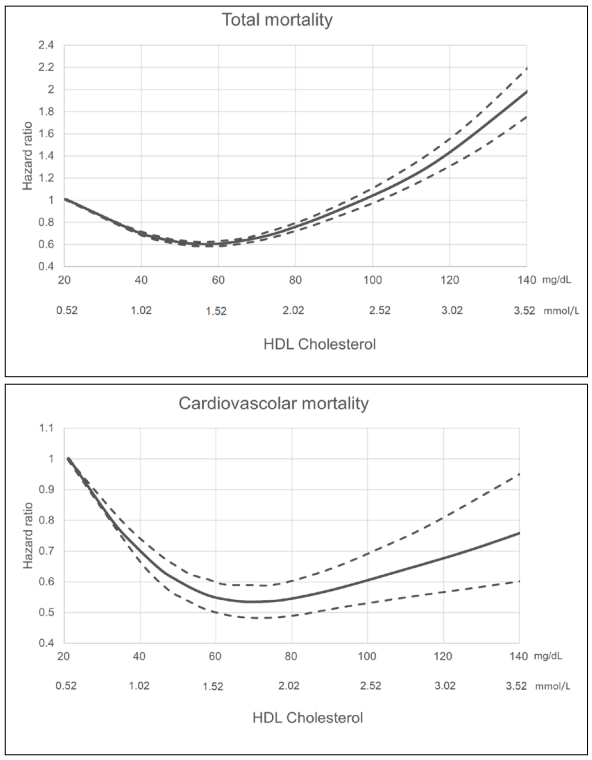

Figure 4
J-curve of total mortality (upper panel) and cardiovascular mortality (lower panel) versus HDL-cholesterol, with trough mortality value at 54-58 mg/dL (1.40-1.50 mmol/L), in the meta-analysis by Zhong et al (16) The solid line represents the estimated value, the dashed lines the 95% confidence interval. From (16), modified.

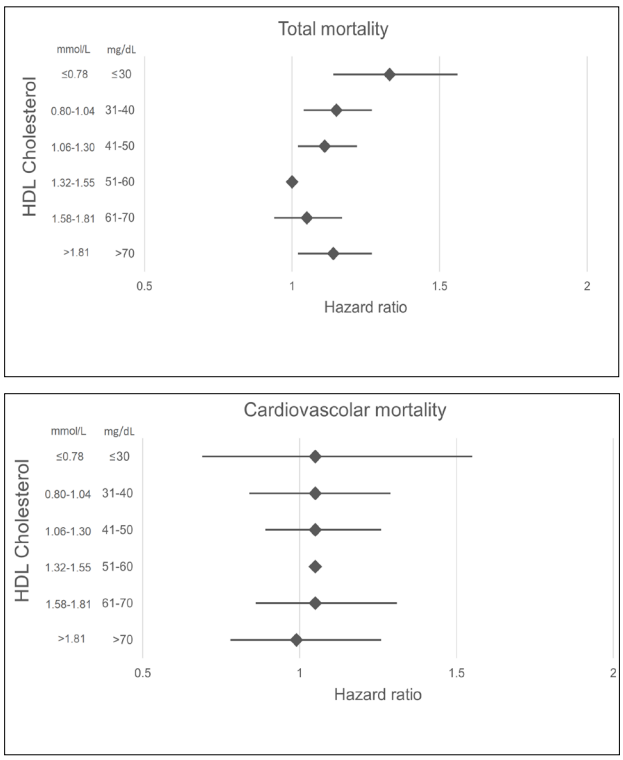

Figure 5
Total mortality (upper panel) and cardiovascular mortality (lower panel) in the analysis of NHANES data (17), compared to the reference value of HDL-cholesterol 51-60 mg/dL (1.32-1.55 mmol/L). Also in this series there is an increase in total mortality for very low and very high values of HDL cholesterol. Data adjusted for age, gender, ethnicity, education, smoking, body mass index, systolic blood pressure, eGFR, caloric intake, total cholesterol, comorbidities (hypertension, diabetes, cardiovascular disease, cancer), and drug therapy (antihypertensives, hypoglycemics, lipid-lowering, platelet aggregation inhibitors).
From (17), modified.

higher mortality in Caucasians with low HDL-C, while for HDL-C >70 mg/dL (1.81 mmol/L) mortality was not significantly increased in non-Caucasians (HR 1.06; 95%CI 0.73-1.54), nor in Caucasians (HR 0.94; 95%CI 0.68-1.30). Cardiovascular mortality did not show a significant interaction for the presence or absence of cardiovascular disease (p=0.07), nor was there a trend towards the difference for HDL-C values >70 mg/dL (1.81 mmol/L) between the presence (HR 0.97; 95%CI 0.62-1.53) or absence (HR 0.99; 95%CI 0.74-1.33) of cardiovascular disease.

There was no difference even after stratification by treatment with lipid-lowering drugs (p=0.10).

Role of physical activity

The effect of physical activity on HDL-C levels has been documented in a recent meta-analysis (18). Concerning high-density lipoproteins (LP-HDL), it is now clear that serum values of the lipid and apolipoprotein components of this lipid particles increase in parallel with the level of physical activity (19). In fact, professional endurance sport athletes frequently have resting HDL-C values well above normal (even very high) (20), and a concomitant increase in the serum concentration of lipoprotein(a), a highly atherogenic lipid fraction, which actively participates in the pathogenesis of atherosclerosis (21).

A recent meta-analysis published by Blond et al. (22) concluded that the risk of death appears to be lower in physically active individuals, even in those with levels of physical activity well above those commonly recommended. However, this result is not in keeping with one of the largest prospective studies conducted to date, that analyzed the relationship between physical activity and mortality, the Copenhagen City Heart Study (23). The trial included 1 098 healthy joggers and 3 950 non running control subjects, followed for over 10 years, underlying the existence of a U shaped pattern (therefore comparable to that shown between HDL-C and mortality) between jogging intensity and total mortality. Subjects practicing jogging "particularly strenuous" (>4 hours/week, at speed >7 miles/hour) had a substantially greater risk of total mortality (HR, 9.08; 95% CI: 1,87-44.01) compared to those who practiced lighter forms of jogging.

Even the data on professional athletes appear contradictory, as some studies have highlighted a higher risk of death in these subjects (24,25), while this association was not confirmed in other studies (26,27).

Further studies have shown how (too) high levels of physical activity can annul the benefit of physical activity,

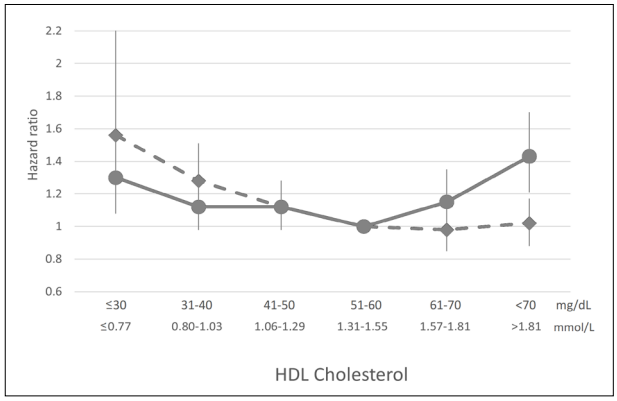

Figura 6

Total mortality in the analysis of NHANES data (17) according to gender, compared to the reference value of HDL cholesterol 51-60 mg/dL (1.32-1.55 mmol/L). Males (n=18 286) solid line, Females (n=18 120) dashed line. The increase in mortality with high HDL cholesterol values is not present in the curve relating to females. From (17), modified.

completely reversing the positive trend that characterizes intermediate levels of physical exercise intensity (28,29). A recent meta-analysis (30) demonstrates how the increase in the frequency of running sessions (from 1 to 6 per week) and the increase in metabolic expenditure during training (from <500 to about 3 000 metabolic equivalents per minute per week) produce a gradual reduction on the risk of total mortality of the benefit deriving from running. It is interesting to note that this meta-analysis shows also that running habitually for 4-5 hours completely revokes the benefit of running; this is evident also when the duration of the sessions is shorter.

Therefore, a crucial question seems to emerge from these premises: can the higher risk of mortality observed in some categories of "super sportsmen" be in some way connected to their characteristically (extremely) high HDL-C values? None of the studies on elevated HDL-C levels considered physical activity or its intensity as covariates according to which to adjust the statistical analysis.

REMARKS ON THE EPIDEMIOLOGICAL DATA

The data reported evidence a fact not been sufficiently emphasized by the scientific literature up to now: an increased risk of death in subjects with high levels of HDL-C. How common is the phenomenon? HDL-C values considered to be elevated (70-80 mg/dL; 1.81-2.07 mmol/L) are more common (6.9%) in the UK Biobank's without ischemic heart disease (15), and in the unselected series of the NHANES study (17) (12.8%), while it appeared between 1.6-1.8% in patients with ischemic heart disease (1). However, there are no data on concomitant therapy that could attribute this difference to lipid lowering treatment.

This mortality trend seems to represent an aspect to be considered, especially in primary prevention. Not the entire population, however, appears equally susceptible, but there is a constant and significant difference based on the gender. In fact, data on female do not evidence a significant increase in mortality either in primary prevention (15), or in studies of non-selected populations (17), or even in the presence of ischemic heart disease (1). The increase in mortality appears significant in males, even if high HDL-C values are less frequent in males in all the series.

Multivariate analyses ruled out a clear role of alcohol consumption in influencing mortality in subjects with high HDL-C values, although the percentage of frequent consumption tends to increase in parallel with HDL-C values. One should remember that the definition of frequent drinking differs between the studies; in the case of the UK Biobank it was defined as consumption greater than or equal to 3 times a week (1,15), while in the EmCAB it was defined as consumption of 8 or more alcoholic beverages a week (1). The definition is an issue: actually, in the UK Biobank subjects frequent consumption is significantly more common in women (15). The definition of frequent consumption could then fail to identify the phenomenon of "binge-drinking" (especially on weekends), and to classify the daily consumption of modest amount as frequent instead. Mortality in subjects with high HDL-C, was increased regardless of whether they were frequent drinkers or not in this series. Also, in the study by Liu et al. in primary prevention (15) the mortality appeared significantly increased, regardless of whether or not they were frequent drinkers. It therefore appears difficult to attribute a causal role to alcohol consumption.

Concerning physical exercise, many clinical studies, guidelines and recommendations have included physical activity programs aimed at producing improvements in the patient's physical fitness and/or cardiovascular rehabilitation, on the assumption that the resulting increase in LP-HDL is always beneficial (31,32).

Nonetheless, given the enigmatic and intriguing relationship between high HDL-C values, high levels of physical activity and increased risk of mortality, further clinical studies are required, aimed at investigating the real biological interaction between HDL-C and demographic/environmental factors (certainly including diet and physical activity), in the modulation of the mortality risk within the different serum concentrations (low and high) of HDL-C.

Another aspect that contributes to complicate the interpretation of these data is the fact that for high HDL-C values the total mortality clearly increases more than the cardiovascular mortality.

In the meta-analysis by Zhong et al. (16), the J shaped curve at the HDL-C threshold value of 80 mg/dL (2.07 mmol/L) shows HR values for total mortality significantly increased over the trough, while for cardiovascular mortality there is only a modest increase over the minimum HR.

The CANHEART study (33), which is included in the aforementioned meta-analysis, is the only to report cause-specific mortality data, standardized by age. The total mortality in the total male population is 8.1% (95%CI 7.9-8.3%) and increases to 9.2% (95%CI 7.9-10.7%) for HDL-C >80 mg/dL (2.07 mmol/L); cardiovascular mortality in the total population is 2.2% (95%CI 2.2-2.3%),

but drops to 1.8% (95%CI 1.2-2.5%) for high HDL-C. On the other hand, total mortality in women decreases between the total series and the group with high HDL-C, respectively 6.6% (95%CI 6.4-6.7%) and 5.8% (95%CI 5.2-6.4%), and the same occurs for cardiovascular mortality, respectively 1.9% (95%CI 1.8-1.9%) and 1.7% (95%CI 1.4-2.0%).

When considering what part of total mortality is attributable to cardiovascular mortality, the analysis of data from studies by Liu et al. on subjects with ischemic heart disease (1) and in its absence (15), and from the study by Huang et al. on subjects at risk (17), evidences that in correspondence to high values of HDL-C, the total mortality increases, but the portion represented by cardiovascular mortality does not increase or even decreases significantly (Figure 7). The U-shaped pattern of total mortality is more evident in patients with ischemic heart disease (Figure 7, panels A) (1), in which a 20% reduction in the percentage attributable to cardiovascular mortality is observed (right panel), with increasing HDL-C values. The trend is similar in subjects without ischemic heart disease (Figure 7, panels B) (15) with a lower percentage of cardiovascular deaths, but with a 14% reduction with increasing HDL-C values (right panel). In the unselected series of the NHANES study (17) (Figure 7, panels C) the U shaped pattern is evident, but the reduction in percentage of cardiovascular mortality with increasing HDL-C values is only 2% (right panel), probably because in this series the percentage of cardiovascular mortality is lower than that of subjects without ischemic heart disease (Figure 7, panels B).

It hence appears that the increase in mortality due to high HDL-C values would not be supported by an increased cardiovascular mortality. The interpretation is further complicated by the observation of an increased cancer mortality in the presence of high HDL-C values (16,17).

POSSIBLE PATHOGENIC MECHANISMS

The identification of the possible pathogenetic mechanisms underlying this surprising association (at least according to the historical paradigms of the relationship between HDL-C and mortality) does not appear simple or univocal. It probably requires a detailed analysis of the biological and environmental variables that most frequently can be associated to elevated HDL-C values. Moreover, it is not always possible to understand whether HDL-C has a causal role or rather represents only an epiphenomenon (e.g., a bystander) of a confounding variable. The association between high values of this lipid fraction and mortality risk remains surprising, but perhaps it is not entirely unexpected (16,17).

Role of structure and function of HDL lipoproteins

The LP-HDL class is highly heterogeneous and contains sub-populations of particles of different size, lipid and lipoprotein composition. These sub-populations are in dynamic equilibrium, with continuous exchanges and acquisitions of lipid and apolipoprotein components during their metabolic cycle (34). The laboratory measurement of the two main components, lipid (HDL-C) and apolipoprotein (apo A-I) of LP-HDL represents a rather approximate estimate of the main function of these lipoproteins that is linked to the reverse transport of cholesterol (RCT) (from peripheral cells to the liver and its excretion) and, therefore, to its anti-atherogenic role (35).

Furthermore, it must be considered that the parameter measured in the laboratory is mainly the HDL-C (apo A-I is measured very rarely), and that the main clinical studies, starting with the Framingham Heart Study of the 1960s (36), have almost exclusively included this measure in their evaluation. However, the progress of knowledge on the complicated metabolism of LP-HDL has led to believe that the view that links the plasma concentration of HDL-C to the efficiency of the RCT is probably too simplistic (35). The RCT is a somewhat complex mechanism that involves the transformation of nascent discoidal LP-HDL into mature spherical LP-HDL, with acquisition and transfer of lipid and protein constituents between the lipoprotein classes in a dynamic equilibrium. It is therefore conceivable that in individuals with particularly high HDL-C plasma concentrations, the composition and the functionality of LP-HDL may be compromised through mechanisms that are still unclear at the moment, thus limiting the ability of these particles to promote the efflux of cholesterol from peripheral cells (35). Based on this hypothesis, Rohatgi et al. (37) postulated and preliminarily demonstrated that the measurement of LP-HDL function could be a more reliable marker than the measurement of plasma HDL-C for predicting cardiovascular events. However, this is a measure that is not currently available in clinical laboratories.

A further property of LP-HDL that could explain the association of elevated HDL-C levels and mortality is its effects on inflammation (38). Apo A-I is one of the main effectors of RCT (37); however, being exposed on the surface of LP-HDL, it can be easily damaged by oxidative stimuli and thus act as a limiting factor within the RCT. Individuals with high HDL-C values consequently also show high values of apo-A-I which, if damaged, exerts a more accentuated limitation on the cholesterol efflux.

The role of genetics

Genetic risk could be implicated, but this hypothesis seems unlikely, since in the study by Liu et al (1), the GRS-adjusted model did not significantly modify the relationship between elevated HDL-C levels and mortality. Studies of Mendelian genetics from a few years ago (39,40) confirm the hypothesis that high HDL-C values do not confer cardiovascular protection.

The first study (39) evaluated 14 Single Nucleotide Polymorphisms (SNPs) exclusively related to plasma HDL-C concentrations, in a significant number of myocardial infarction cases and controls. It concluded that the tested genetic mechanisms that increase HDL-C do not appear to decrease the risk of myocardial infarction. On the other hand, the GRS of SNPs linked to LDL cholesterol is instead associated with an increased risk,

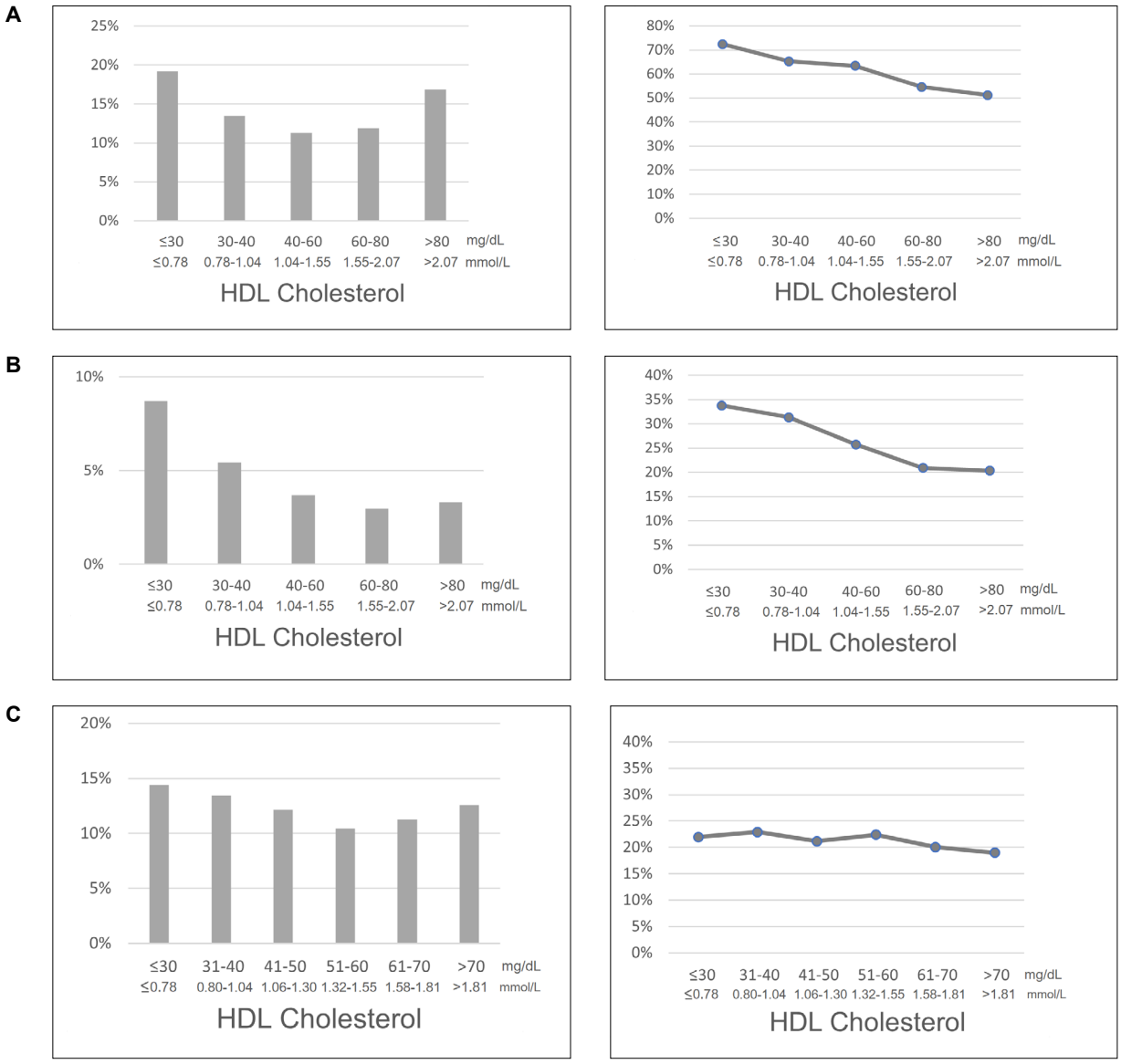

Figure 7
Rate of cardiovascular mortality on total mortality as HDL cholesterol values increase. Left panels: total mortality according to HDL cholesterol value. Right panels: Percentage of total mortality due to cardiovascular mortality.
A: patients with ischemic heart disease (ref 1).
B: subjects without heart disease (ref 15).
C: NHANES (National Health and Nutrition Examination Surveys) case series (ref 17).
The U-shaped pattern of total mortality is more evident in patients with ischemic heart disease (panels A); it is also present in subjects without ischemic heart disease (panels B) (15) with a lower percentage of cardiovascular deaths. In the unselected series of the NHANES study (17) (panels C) the U-shaped pattern is present, but less marked, probably due to low cardiovascular mortality
(from references 1,15,17: processing of data from the original works).

(as expected), thus confirmin the validity of the model used by the Authors. The second study (40) presents very similar results, using for comparison the SNPs linked to the plasma concentrations of triglycerides.
Also in this case the genetic analysis supports the hypothesis that high concentrations of triglycerides are a cardiovascular risk factor, while concerning HDL-C, the mechanism is not evidenced with sufficient certainty

VARIABILITY OF HDL CHOLESTEROL MEASUREMENT

Considering the available evidence and related considerations, it is probably necessary to identify a range of HDL-C values that can be considered “beneficial”. The analysis of the J or U curves (16,20) suggests a narrow range of values, which can be hypothesized between 60 and 80 mg/dL (1.55-2.07 mmol/L), as analyzed in more detail in the following paragraph.

Nonetheless, this hypothesis is based on the assumption that the laboratory measurement of the parameter is sufficiently precise and accurate. The verification that this is actually the case in the daily laboratory practice is entrusted to the analysis of the data available from EQA programs. In fact, the examination of recent data concerning this parameter provided by the Biomedical Research Center for Quality in Laboratory Medicine (CRB), a specialized center of the Veneto Region, provides a panorama that is not always adequate. Table 1 presents the analytical quality data (CVa) of the CRB EQA for HDL-C for the years 2020 and 2021, compared with the analytical performance specifications defined by European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) on the basis of the biological variability of the parameter (41). The CVa values of the various analytical systems adopted are not particularly satisfactory. Some analytical systems present CVa% close to the "minimum" requirement (4.3%) (systems A, B, F), others do not even reach the minimum goal (C), some are rather far from it (D and E). None of the analytical systems reaches the "desirable" goal (2.9%) and even less the “optimal” goal (1.4%) (Table 1). This situation characterizes not only Italy; as proven by an interesting work published a few years ago (42) related to an EQA exercise carried out in Europe using fresh (commutable) samples. The study demonstrated that none of the 5 participating countries (Italy, Holland, Portugal, United Kingdom, Spain) met the desirable performance specification for the total allowable error, which includes accuracy and precision (41,42).

This analytical quality of HDL-C measurement makes the interpretation of HDL-C values uncertain when we would need to compare them with a rather narrow range of values, as suggested by the evidence available today.

It is also necessary to observe that not even in the presence of a single cut-off value could this analytical situation be defined as acceptable. Laboratories, manufacturers and regulatory agencies are hence prompted to take further and stronger efforts for improving the analytical quality of HDL-C in the near future.

FINAL CONSIDERATIONS

Considering the available data, which confirms the existence of a J-shaped, if not even a U-shaped, association between HDL-C and mortality risk, it seems legitimate to raise reasonable doubts regarding the indications included in some recent guidelines on cardiovascular risk, which advocate a general increase in HDL-C values (mainly through physical activity and lifestyle changes). Some highly visited websites of undisputed scientific value such as those of the Mayo Clinics (43) or the Cleveland Clinics (44), report the indication that the desirable value of HDL-C should be *"60 mg/dL (1.55 mmol/L) or greater"* or “*ideally greater than 60 mg/dL" (1.55 mmol/L)"*. The website of the Italian Istituto Superiore di Sanità (ISS) reports the indication that the HDL-C value is desirable when it is "*equal to or greater than 50 mg/dL" (1.30 mmol/L)"* (45).

Regardless of the heterogeneity of the indications on the cutoff value of HDL-C, which in itself represents a contradiction, in our opinion these indications should be revised, for example by specifying (as for LDL cholesterol) a range of "desirable values".

In this case it might be reasonable to suggest a desirable range of HDL-C values between 60-80 mg/dL (1.55-2.07 mmol/L), which could then replace the generic, obsolete and now perhaps incongruous indication of "equal to or greater" than a given minimum value.

It would therefore be desirable to plan further studies analyzing in more detail the risk linked to excessive physical activity or to the intake of high doses of alcohol in modulating the association between HDL-C and mortality. Such studies could also clarify whether high HDL-C values directly support an increased mortality, or whether they may be confounding variables that represent an indicator of different mechanisms, on which the data available do not allow us to draw any definitive conclusion so far.

Table 1

Year	Instruments (CVa%)					
	A	B	C	D	E	F
2020	4.96	4.19	3.79	6.54	12.67	2.98
2021	3.38	3.52	4.78	8.04	11.13	4.20

The analytical performance specifications established by EFLM (ref 41) are: minimum 4.3%; desiderable 2.9%; optimum 1.9%.
CVa, analytical coefficient of variation.

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CONFLICT OF INTEREST

None.

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