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Usefulness of Serum Bilirubin and Cardiorespiratory Fitness as Predictors of Mortality in Men

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Abstract

Elevated serum bilirubin has been suggested to reduce the risk of mortality. Cardiorespiratory fitness (CRF) has also been reported to have inverse association with all-cause and cardiovascular disease (CVD) mortality. The association between serum bilirubin, all-cause and CVD mortality and the effect of CRF on the observed association was investigated. A total of 1279 men, ages 30-82 years old, who underwent baseline medical examinations during 1974 to 1997 at the Cooper Clinic in Dallas, Texas. During an average of 17 years follow-up, 698 men died, with 253 deaths due to CVD (36%). Men in the highest bilirubin quartiles had significantly lower risk of all-cause mortality compared to men in the lowest quartiles (p for trend=0.0043), after adjusting for age and examination year. This inverse association remained significant after further adjustment for known confounders (p for trend=0.0018). Additional adjustment for treadmill time attenuated the association (p trend=0.0090). Similar patterns of association were observed between serum bilirubin quartiles and CVD mortality. CRF was inversely associated with all-cause mortality (p for trend < .0001) after adjusting for age and examination year. This inverse association also was observed after further adjusting for known confounders (p for trend=0.0004). After additional adjustment for serum bilirubin, the association between the CRF and all-cause mortality remained significant (p for trend = 0.0012). All-cause mortality and CVD mortality were significantly lower among men in the moderate to high fit quartiles in both the low and high bilirubin groups. In Conclusion both serum bilirubin level and CRF level were strongly and independently associated with all-cause and CVD mortality.

MeSH Keywords

serum bilirubin; cardiorespiratory fitness; cardiovascular disease; men; mortality

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Serum bilirubin has been shown to have potent antioxidant properties whereas, cardiorespiratory fitness (CRF) has been suggested as a way to help maintain and promote the antioxidant defense capabilities of the body against oxidative stress.¹⁻³ In recent years mild hyperbilirubinemia has been reported to reduce the risk of mortality.^{2,4-6} The inverse association between CRF, all-cause and cardiovascular disease (CVD) mortality has also been well documented in the literature.^{7,8} The aim of this study was to investigate the association of elevated serum bilirubin levels with all-cause mortality and CVD mortality in men, and to examine the impact of cardiorespiratory fitness on the observed association.

Methods

This study is based on data from a prospective nested case-control study obtained from the Aerobics Center Longitudinal Study (ACLS).⁶ In the original study, case subjects were those who died from any cause during the follow-up period (up until the end of 1997), and control subjects for cases were selected randomly from among survivors who met the matching criteria for age (± 5 years). For the current study, we used the original study sample and followed up for mortality through 2003 using an updated mortality database.

The present study population consisted of 1279 adult men between the ages of 30 and 82 who underwent baseline medical examinations during 1974 to 1997 at the Cooper Clinic in Dallas, Texas. Medical evaluation was performed after an overnight fast of at least 12 hours. Participants came to the clinic for periodic health examinations and counseling regarding diet, exercise, and other lifestyle factors associated with increased risk of chronic diseases. Many of the study participants were referred by their employer for the examination, some were referred by their personal physician, and others were self-referred. Participants were excluded from the study if they had history of CVD or cancer at baseline (n=98), liver problems (fatty food intolerance, gallbladder trouble, jaundice, hepatitis, or cirrhosis) (n=5), body mass index (BMI) ≤ 18 kg/m² (n=1), were unable to achieve 85% of their age-predicted maximal heart rate (220-age) on a graded exercise treadmill test (n=74), or were followed for < 1 year (n=32). The majority of the study population was white, well-educated and from middle to upper class socioeconomic strata. Participants provided written informed consent to participate in the study, and the study protocol was approved annually by the Cooper Clinic institutional review board.

The examination included fasting blood chemistry analysis, personal and family health history, anthropometry, resting blood pressure, electrocardiogram (ECG), and a maximal graded exercise test, performed during a single clinic visit. The baseline procedures have been described elsewhere.⁸ Briefly, body weight and height were measured using a standard physician scale and stadiometer and BMI was computed from weight and height (kg/m²). Resting blood pressure was measured after a brief period of sitting quietly, using auscultatory methods with a mercury sphygmomanometer at the first and fifth Korotkoff sounds. Serum plasma and whole blood samples were drawn from antecubital vein and were analyzed for lipids and glucose using automated bioassays in accordance with the Centers for Disease Control and Prevention (CDC) Lipid Standardization Program. Total serum bilirubin from non-hemolyzed serum was analyzed with a commercial calorimetric method using diazotized sulfanilic reagent. Diabetes mellitus was defined as fasting plasma glucose level ≥ 126 mg/dl, a history of physician-diagnosed diabetes mellitus, or current therapy with insulin. Hypertension was defined as resting blood pressure $\geq 140/90$ mm Hg or a history of physician-diagnosed hypertension. Information regarding smoking habits (never, former or current), alcohol intake (number of drinks per week, correcting for alcohol content, where 1 unit of alcohol was defined as 12 oz. of beer, 5 oz. of wine, or 1.5 oz. of hard liquor), and parental history of CVD was obtained using a standardized questionnaire. Abnormal resting and exercise ECG responses included rhythm and conduction disturbances

and ischemic ST-T wave abnormalities, described in detail elsewhere.⁹ Previously, we found 90% agreement between the ECG interpretation recorded in our database and that of a group of 3 physicians who read a random sample of 357 patient records. Cardiorespiratory fitness was determined based on maximal treadmill time and was calculated using the modified Balke protocol.¹⁰ Patients were encouraged to give a maximal effort, and the test endpoint was volitional exhaustion or termination by the physician for medical reasons. The total time (minutes) was used as an index of aerobic power. Time spent on the treadmill in this protocol correlates highly ($r=0.92$) with maximal oxygen uptake in men.¹¹ The speed and elevation of the final minute of the treadmill test was used to convert treadmill performance to metabolic equivalents (METs). One MET is an oxygen uptake of 3.5 ml/kg per minute, the energy expenditure at a resting state.

Participants were followed from the date of their baseline measurement until their date of death or December 31, 2003. All-cause mortality and cardiovascular mortality were determined from the National Death Index report or by review of death certificates in the decedent's state of residence. CVD mortality was defined using International Classification of Diseases, Ninth Revision (ICD-9) codes 390 to 449.9, before 1999 and the Tenth Revision codes 100-178 during 1999-2003.

Baseline characteristics were summarized across bilirubin quartiles. Trends in covariates over bilirubin were assessed using *F* tests. Participants were classified into four groups based on quartiles of bilirubin levels and age-specific treadmill time (20-39, 40-49, 50-59, ≥ 60 years-old). For the joint analysis, participants were classified into low bilirubin (Q1-Q3) or high bilirubin (Q4), and low fit (Q1) or high fit (Q2-Q4) groups based on our results that showed a lower mortality risk in the highest bilirubin quartile and a lower all-cause mortality risk in quartiles 2 to 4 of treadmill time. The decision to use quartiles of CRF in this study was made in order to be consistent with the bilirubin classification.⁵ Cox regression analysis was used to estimate relative risk (RR) and 95% confidence intervals (CI) of all-cause mortality and CVD mortality according to total serum bilirubin level (deaths per 10,000 person-years of follow up). Multivariable analysis included the following confounders: age (years), examination year, BMI, smoking status (never, past or current smoker), alcohol intake (number of drinks per week), presence or absence of abnormal ECG, hypertension, diabetes, total cholesterol, a family history of CVD, and maximal treadmill time or bilirubin levels. All *P* values are two-sided and $P < 0.05$ was regarded as statistically significant. All analyses were performed using SAS statistical software, version 9.2 (SAS Inc, Cary, NC).

Results

In this study population, 698 men died during follow-up, with 253 deaths due to CVD (36%). The mean (SD) follow-up period was 17 (9) years, and the mean age (SD) was 52 (10). The baseline characteristics of the 1279 participants included in this study are described in Table 1. The mean (SD) serum bilirubin in each quartile was 0.36 (0.06), 0.55 (0.05), 0.75 (0.05) and 1.12 (0.27), respectively, and ranged from 0.10-2.60 mg/dl. The correlation between bilirubin level and treadmill time in this population was $r=0.07$ ($P=0.009$).

Table 2 shows the RR of mortality across quartiles of serum bilirubin. After adjusting for age and examination year (model 1), total serum bilirubin level was inversely associated with all-cause mortality (p for trend=0.0043), and men in the fourth quartile (Q4) had significantly lower risk of all-cause mortality compared to men in the first and second quartiles (Q1-Q2). This inverse association remained significant after adjusting for smoking status, alcohol intake, BMI, total cholesterol, hypertension, diabetes, abnormal ECG, and

parental CVD (model 2) (p for trend=0.0018). After additional adjustment for treadmill time, the inverse association between bilirubin quartiles and all-cause mortality was attenuated (p for trend=0.0090). However, men in the highest bilirubin quartiles still had a significantly lower risk of all-cause mortality. Similar patterns of association were observed between serum bilirubin quartiles and CVD mortality (Table 2). After excluding men with total serum bilirubin level > 1.00 mg/dl, who were considered to have Gilbert's syndrome which causes elevation of bilirubin, the associations between total serum bilirubin level and all-cause mortality and CVD mortality in men were comparable (data not shown).

Table 3 presents the results of the relative risk of mortality in the age-specific CRF quartiles. After adjusting for age and examination year (model 1), the age-specific CRF had a significant inverse association with all-cause mortality (p for trend <0.0001), with men in the Q2 to Q4 having significantly lower all-cause mortality compared to men in Q1. This association with all-cause mortality was observed after further adjusting for smoking status, alcohol intake, BMI, total cholesterol, hypertension, diabetes, abnormal ECG, and parental CVD (model 2) (p for trend <0.0004). After additional adjustment for bilirubin level (model 3), the association between CRF and all-cause mortality remained significant (p for trend=0.0012). A similar pattern of association was observed between CRF quartiles and CVD mortality (p for trend <0.05).

Table 4 shows the joint association of bilirubin level and CRF after adjusting for age, examination year, smoking status, alcohol intake, BMI, total cholesterol, hypertension, diabetes, abnormal ECG, and parental CVD. All-cause mortality and CVD mortality were significantly lower among men in Q2-Q4 in both the low (RR=0.70, 95% CI 0.57-0.85; RR=0.70, 95% CI 0.51-0.96) and high bilirubin groups (RR=0.55, 95% CI 0.43-0.70; RR=0.46, 95% CI 0.31-0.69).

Discussion

The findings of our current study are consistent with previous reports of the association between total serum bilirubin level and all-cause mortality in men, which found a lower risk of mortality in the highest bilirubin quartiles.^{5,6} Most of the studies that have examined the association between serum bilirubin level and CVD disease did not include death as an endpoint.^{1,2,4,12-14} and furthermore, none of the previous studies took CRF, a strong and independent mortality predictor, into account in their analyses.

Our results showed that CRF had a strong inverse association with all-cause mortality and a significant association with CVD, adjusted for age and examination year (model 1). These associations remained significant after adjusting for possible confounders and bilirubin levels (models 2 and 3). These results support our earlier findings,^{7,8,15} as well as findings of other groups on physical activity and mortality,^{16,17} adding to the existing body of evidence recognizing the importance of CRF in reducing the risk of all-cause and CVD mortality in men.

To our knowledge, this is the first study to examine the joint association between total serum bilirubin and CRF with respect to all-cause and CVD mortality in men. Our findings suggest that, after adjusting for known confounders (i.e. age, examination year, smoking status, alcohol intake, BMI, cholesterol, hypertension, diabetes, abnormal ECG, and parental CVD), men in the moderate-high fit (Q2-Q4) quartiles had a lower risk of all-cause and CVD mortality regardless of their total serum bilirubin level, when compared to men in the low-fit group. If confirmed by future studies, the above findings may have important implications as they suggest that leading a physically active lifestyle that results in moderate

to high levels of cardiorespiratory fitness may reduce the risk of premature death, regardless of serum bilirubin levels.

The mechanisms for a causal association between serum bilirubin and all-cause or CVD mortality are not yet well understood. One of the proposed mechanisms is the protective antioxidant properties attributed to serum bilirubin.^{18,19} Earlier studies have suggested that bilirubin exerts this effect through two pathways; the inhibition of lipid oxidation, especially LDL, which is otherwise highly susceptible to oxidation and/or through the inhibition of vascular endothelial activation, which is seen as an important step in the formation of lipid-rich foam cells leading to the formation of atherogenic plaque.²⁰⁻²³

In a more recent study, Sedlak & Snyder²⁴ suggested that mild to moderate elevation of serum bilirubin levels is associated with better outcomes in diseases involving oxidative stress. Vitek and colleagues²⁵ also reported findings suggesting that mild elevation of serum bilirubin levels may exert a protective action predominately through scavenging ROS (reactive oxygen species) and that bilirubin exerts approximately 10% of the total antioxidant capacity in normobilirubinemic adults. A number of in vitro studies have shown that bilirubin is more efficient than α -tocopherol at inhibiting low-density lipoprotein (LDL) oxidation and have argued that bilirubin is a more efficient protector of human ventricular monocytes than either vitamin C or vitamin E.²⁶

The strengths of this study include the prospective study design, the use of extensive assessment for known clinical risk factors, and the objective measurement of CRF used in the ACLS. However, the results of this study can only be generalized to the population study group (i.e., male, predominately white, and middle to upper class) and further studies are needed to confirm these findings in other population groups.

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Table 1

Baseline characteristics by serum bilirubin levels

	Bilirubin Quartiles (mg/dl)					P for Trend
	Q1 (0.10-0.49)	Q2 (0.50-0.69)	Q3 (0.70-0.89)	Q4 (0.90-2.60)		
Number of patients	225	398	317	339		
Age (years)	51.97 ± 10.1	52.14 ± 10.41	52.12 ± 10.63	52.60 ± 10.60		0.89
Bilirubin (mg/dl)	0.36 ± 0.06	0.55 ± 0.05	0.75 ± 0.05	1.12 ± 0.27		<.0001
Maximal Treadmill time (min)	14.18 ± 5.13	14.80 ± 5.40	15.40 ± 5.10	15.40 ± 4.78		0.02
Maximal METs	9.89 ± 2.42	10.19 ± 2.55	10.47 ± 2.42	10.45 ± 2.23		0.02
Weight (kg)	82.30 ± 12.48	82.53 ± 11.24	81.88 ± 13.01	83.34 ± 13.11		0.50
Height (cm)	177.81 ± 6.04	177.82 ± 6.03	177.91 ± 6.40	178.08 ± 6.49		0.94
Body mass index (kg/ m ²)	25.97 ± 3.15	26.08 ± 3.18	25.82 ± 3.53	26.23 ± 3.55		0.47
Smoking Status						
Never	163 (12.7%)	293 (22.9%)	240 (18.8%)	268 (21%)		0.24
Former	3 (0.2%)	13 (1%)	12 (0.9%)	15 (1.2%)		0.24
Current	59 (4.6%)	92 (7.2%)	65 (5.1%)	56 (4.4%)		0.03
Alcohol intake (drinks/week)	8.60 ± 12.52	8.75 ± 12.61	9.34 ± 11.24	9.94 ± 12.37		0.50
Systolic blood pressure (mmHg)	125 ± 16	127 ± 17	126 ± 16	127 ± 17		0.15
Diastolic blood pressure (mmHg)	82 ± 10	82 ± 10	82 ± 10	83 ± 11		0.37
Fasting glucose (mg/dl)	106.34 ± 24.57	107.24 ± 31.85	103.88 ± 22.13	106.45 ± 28.64		0.42
Total cholesterol (mg/dl)	218.12 ± 41.87	220.11 ± 38.32	219.41 ± 44.83	222.34 ± 37.74		0.65
Hypertension *	83 (6.5%)	174 (13.6%)	141 (11%)	159 (12.4%)		0.13
Diabetes Mellitus †	32 (2.5%)	49 (3.8%)	31 (2.4%)	41 (3.2%)		0.46
Abnormal ECG	34 (2.7%)	73 (5.7%)	48 (3.8%)	67 (5.2%)		0.32
Parental CVD	99 (7.7%)	167 (13.1%)	108 (8.4%)	134 (10.5%)		0.08

* Hypertension was defined as history of physician diagnosis or resting systolic/diastolic blood pressure ≥140/90 mm Hg.

† Diabetes was defined as history of physician diagnosis, use of insulin or measured fasting glucose level ≥126 mg/dL (7.0 mmol/L).

Table 2

Relative risk of mortality by serum bilirubin levels

	Bilirubin Quartiles				P for Trend
	Q1 (0.10-0.49)	Q2 (0.50-0.69)	Q3 (0.70-0.89)	Q4 (0.90-2.60)	
<i>All-cause mortality</i>					
No. of Death	134	225	171	168	
Death Rate *	374	339	310	274	
Model 1 RR (95% CI) [†]	1.00 (reference)	0.91 (0.73-1.12)	0.83 (0.66-1.04)	0.74 (0.58-0.92)	0.0043
Model 2RR (95% CI) [‡]	1.00 (reference)	0.86 (0.69-1.07)	0.83 (0.66-1.04)	0.69 (0.55 - 0.87)	0.0018
Model 3 RR (95% CI) [~]	1.00 (reference)	0.89 (0.71-1.10)	0.86 (0.68-1.08)	0.73 (0.58-0.92)	0.0090
<i>CVD mortality</i>					
No. of Death *	46	84	67	56	
Death Rate	131	127	120	91	
Model 1 RR (95% CI) [†]	1.00 (reference)	0.97 (0.68-1.39)	0.92 (0.63-1.34)	0.69 (0.47-1.02)	0.0446
Model 2RR (95% CI) [‡]	1.00 (reference)	0.83 (0.58 -1.20)	0.88 (0.60-1.29)	0.58 (0.39-0.86)	0.0090
Model 3 RR (95% CI) [~]	1.00 (reference)	0.85 (0.59-1.23)	0.91 (0.62-1.34)	0.61 (0.41-0.91)	0.0217

* Death rate per 10,000 – Pearson Years

[†]Model 1: adjusted for age and examination year[‡]Model 2: adjusted for age, examination year, smoking status, alcohol intake, BMI, total cholesterol, hypertension, diabetes, abnormal ECG, and parental CVD[~]Model 3: adjusted for age, examination year, smoking status, alcohol intake, BMI, total cholesterol, hypertension, diabetes, abnormal ECG, parental CVD, and treadmill time

Table 3
Relative risk of mortality by cardiorespiratory fitness levels

Age-Specific Cardiorespiratory Fitness Quartiles					
	Q1	Q2	Q3	Q4	P for Trend
<i>All-cause mortality</i>					
No. of Death	225	178	143	152	
Death Rate *	486	306	251	249	
Model 1 RR (95% CI) [†]	1.00 (reference)	0.63 (0.52-0.77)	0.52 (0.42-0.64)	0.51 (0.41-0.63)	<.0001
Model 2RR (95% CI) [‡]	1.00 (reference)	0.71 (0.58-0.87)	0.61 (0.49-0.77)	0.68 (0.54-0.86)	0.0004
Model 3 RR (95% CI) [~]	1.00 (reference)	0.73 (0.59-0.89)	0.63 (0.50-0.79)	0.70 (0.55-0.89)	0.0012
<i>CVD mortality</i>					
No. of Death	95	67	43	48	
Death Rate *	176	111	91	90	
Model 1 RR (95% CI) [†]	1.00 (reference)	0.56 (0.41-0.77)	0.37 (0.26-0.53)	0.39 (0.27-0.56)	<.0001
Model 2RR (95% CI) [‡]	1.00 (reference)	0.75 (0.54-1.03)	0.53 (0.36-0.78)	0.70 (0.47-1.05)	0.0170
Model 3 RR (95% CI) [~]	1.00 (reference)	0.77 (0.55-1.07)	0.55 (0.37-0.81)	0.74 (0.49-1.11)	0.0334

* Death rate per 10,000 – Pearson Years

[†]Model 1: adjusted for age and examination year

[‡]Model 2: adjusted for age, examination year, smoking status, alcohol intake, BMI, total cholesterol, hypertension, diabetes, abnormal ECG, and parental CVD

[~]Model 3: adjusted for age, examination year, smoking status, alcohol intake, BMI, total cholesterol, hypertension, diabetes, abnormal ECG, parental CVD, and bilirubin levels

Table 4
Relative risk of mortality by combination of serum bilirubin and cardiorespiratory fitness levels

	Low fit (Q1)		Moderate-high Fit (Q2-Q4)	
	Low Bilirubin (Q1-Q3)	High Bilirubin (Q4)	Low Bilirubin (Q1-3Q)	High Bilirubin (Q4)
<i>All-cause mortality</i>				
No. of Death	179	46	351	122
*RR (95% CI)	1.00 (reference)	0.86 (0.62-1.19)	0.70 (0.57-0.85)	0.55 (0.43-0.70)
<i>CVD mortality</i>				
No. of Death	77	18	120	38
*RR (95% CI)	1.00 (reference)	0.72 (0.43-1.21)	0.70 (0.51-0.96)	0.46 (0.31-0.69)

* adjusted for age, examination year, smoking status, alcohol intake, BMI, total cholesterol, hypertension, diabetes, abnormal ECG, and parental CVD