Serum bilirubin is associated with lung function in a Swiss general population sample

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ABSTRACT Bilirubin is a strong antioxidant. Increased serum levels have been associated with lower respiratory disease and mortality risk. We studied the association of bilirubin with lung function in the Swiss study on Air Pollution and Lung Disease in adults (SAPALDIA) cohort.

Associations between natural logarithmised bilirubin and forced expiratory volume in 1 s (FEV_1), forced vital capacity (FVC), FEV_1/FVC and mean forced expiratory flow between 25%–75% of FVC ($FEF_{25–75\%}$) were tested using multiple linear regression in the whole study population (n=4195) and strata of ever-smoking and high body mass index (BMI, defined by the highest distribution quartile). Associations were retested with single nucleotide polymorphism rs6742078, a genetic determinant of bilirubin.

High bilirubin levels were significantly associated with higher FEV_1/FVC and $FEF_{25–75\%}$ overall. Upon stratification, significant associations persisted in ever-smokers, amounting to 1.1% (95% CI 0.1–2.2%) increase in FEV_1/FVC, and 116.2 $mL \cdot s^{-1}$ (95% CI -15.9–248.4 $mL \cdot s^{-1}$) in $FEF_{25–75\%}$ per interquartile range of bilirubin exposure in smokers with high BMI. Associations were positive but nonsignificant in never-smokers with high BMI. Similarly, rs6742078 genotype TT was associated with increased FEV_1/FVC and $FEF_{25–75\%}$.

Our results suggest a possible protective role of bilirubin on lung tissue, which could be important for prevention and therapy.

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Introduction

Bilirubin is an end-product of heme degradation in the body that has raised considerable interest in research over the last decade [1]. Increased serum bilirubin has repeatedly been associated with decreased risks for coronary artery disease, myocardial infarction, peripheral arterial disease and stroke in retrospective and prospective clinical studies [2, 3]. The evidence for beneficial effects of elevated serum bilirubin was supported by animal and *in vitro* experiments showing antioxidative and anti-inflammatory properties [4].

Beneficial effects of elevated serum bilirubin have also been observed on respiratory health. Higher bilirubin concentrations were associated with lower incidence of lung cancer, chronic obstructive lung disease and lung cancer mortality, as well as height-standardised lung function in previous population-based studies [5–7]. These results suggest that bilirubin might have protective effects in tissues exposed to the outer environment, such as the lungs, possibly by counteracting subclinical inflammation.

Furthermore, serum bilirubin levels were inversely associated with body mass index (BMI) [8], were found to be lower in smokers than never-smokers and decreased with higher smoking duration or intensity [9].

The major genetic determinant of serum bilirubin is UDP-glucuronosyltransferase 1A1 (*UGT1A1*) [10]. The enzyme degrades bilirubin by glucuronidation and allows its subsequent excretion into the bile. Polymorphisms in *UGT1A1* explain 10–30% of serum bilirubin variation [1, 11]. A functional repeat polymorphism in the promoter region underlies Gilbert syndrome, which is prevalent in 5–10% of Caucasians. Compared with the wild-type allele consisting of six thymine-adenosine dinucleotides (TA) on each chromosome, persons homozygous for seven TA repeats have a 70% decreased *UGT1A1* activity [11]. A genome-wide study on bilirubin levels suggested that single nucleotide polymorphism (SNP) rs6742078 near *UGT1A1* is in high linkage disequilibrium with the functional promoter repeat polymorphism, with its T-allele increasing bilirubin concentration [10].

Eight studies attempted to corroborate causality for the observed associations between bilirubin and cardiovascular outcomes using Mendelian randomisation, an instrumental variable analysis method, in which *UGT1A1* variants are analysed to obtain unbiased estimates of bilirubin effects [12–19]. The most recent one on 67 068 healthy controls and 11 686 cases with ischaemic heart disease observed no significant association with genetically elevated bilirubin, even after meta-analysis with the earlier studies [19].

No comparable genetic studies have been conducted in the respiratory field, and the potential role of bilirubin in respiratory health has hardly been assessed. We therefore aimed to study whether bilirubin serum levels were associated with lung function in the general population sample of the Swiss study on Air Pollution and Lung Disease in adults (SAPALDIA) cohort study. The level of lung function is an early pre-clinical marker of underlying pathological lung processes such as inflammation and tissue remodelling. We *a priori* tested whether the relationship between bilirubin and lung function was altered by increased vulnerability arising from active tobacco smoking or subclinical inflammation related to excessive body weight. To assess causality for observed bilirubin associations, we studied the association of variant rs6742078 near *UGT1A1* with lung function in a subset of the population.

Methods

Design and study population

SAPALDIA is a cohort study recruiting adults aged 18–60 years from eight Swiss communities. The SAPALDIA methods have been described in detail previously [20]. The present study is based on 4195 participants attending both examinations, with lung function testing, and with complete data on smoking exposure and bilirubin measurement (fig. 1).

Written informed consent was obtained from all study participants, and the study was approved by the Swiss Academy of Medical Sciences, as well as the respective regional ethics committees.

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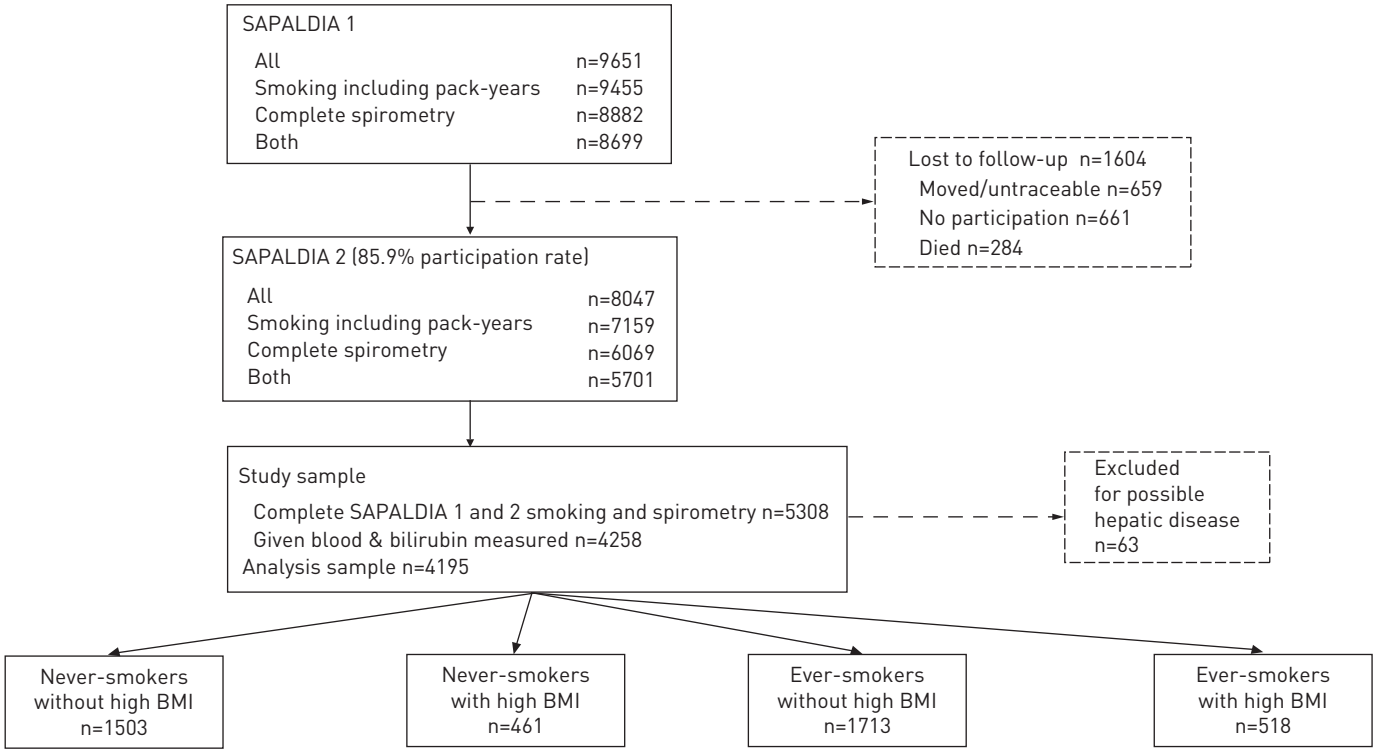

FIGURE 1 Selection of study participants. High body mass index (BMI) is defined as being in the highest quartile of the observed BMI distribution (BMI $\geqslant$28.36 kg·m^{-2}). SAPALDIA: Swiss study on Air Pollution and Lung Disease in adults.

Health questionnaire

All study participants underwent an interview on smoking behaviour, exposure to second-hand smoke, occupational exposures, pre-existing respiratory, allergic and cardiovascular diseases and symptoms, as well as socioeconomic factors.

Education comprised highest attained school degree until follow-up examination and was grouped into three categories: low (primary school only), medium (high school) and high (university or college degree) educational level.

Never-smokers were defined as having smoked <20 packs of cigarettes or 360 g of tobacco during their lifetime. Former smokers had quit smoking at least 1 month before examination, and current smokers reported active smoking at the time of interview. In ever-smokers, pack-years of smoking were calculated by dividing the number of cigarettes per day by 20, giving cigarette packs per day, which were multiplied by years of exposure.

Pre-existing cardiopulmonary disease was defined as self-declaration of heart disease, lung emphysema or chronic bronchitis, or use of health services for respiratory problems during the 12 months prior to follow-up examination. The detailed questions are given in the online supplementary material.

BMI and spirometry

Participants wore no shoes or heavy clothes for the measurement of weight and height. BMI was calculated by dividing weight (in kilograms) by the square of height (in metres).

Lung function testing was done using Sensormedics model 2200 (Sensormedics devices, Yorba Linda, CA, USA). Devices were calibrated daily, and comparability between devices was checked. Measurements were conducted according to the protocol of the European Community Respiratory Health Survey. Participants performed three to eight forced expiratory manoeuvres while sitting in an upright position. Manoeuvres had to comply with American Thoracic Society quality criteria [21], and at least two acceptable values for the forced expiratory volume in 1 s (FEV_1) and forced vital capacity (FVC) were recorded. The ratio of FEV_1/FVC was derived from measurements of the same manoeuvre. Mean forced expiratory flow at 25–75% of FVC ($FEF_{25–75\%}$) was recorded from the curve displaying the highest sum of FEV_1 and FVC.

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Biomarkers

Whole blood samples were collected in 2002, and serum blood markers were measured using a Modular P Autoanalyser (Roche Diagnostics, Rotkreuz, Switzerland). Total bilirubin was determined by the diazo method [22]. Liver enzyme activities were determined by enzymatic ultraviolet (UV) tests (including pyridoxalphosphate) for alanine aminotransferase (ALT) and aspartate aminotransferase (AST), and by photometric test for γ-glutamyl transferase (GGT). Coefficients of variation ranged from 1–3%.

Bilirubin values >17 μmol·L^{-1} (upper normal value) with levels of ALT, AST or GGT equalling or surpassing the double of the upper normal value (50, 52 and 66 U·L^{-1}, respectively) were set to missing due to possible liver disease.

Genotype data for rs6742078 near UGT1A1 was acquired in the genome-wide GABRIEL asthma study for 982 nonasthmatic individuals [23, 24]. Being nonasthmatic was defined as neither self-reported nor doctor-diagnosed asthma using a standardised questionnaire [20]. Genotyping details are available in the online supplementary material.

Definition of susceptible groups

Two susceptibility conditions were considered regarding lung function. The first comprised ever-smoking. The second included high BMI because of the associated subclinical inflammation, which might interfere with the protective effects of bilirubin. For different reasons, this condition was defined statistically by using the highest quartile of the BMI distribution (BMI $\geqslant$28.36 kg·m^{-2}) as threshold. First, waist circumference, more closely related to subclinical inflammation, was not available; and, secondly, categorising on the highest quartile was reasonable in absence of a validated BMI threshold to capture inflammation, as the common overweight definition of BMI $\geqslant$25 kg·m^{-2} was met by a large proportion of our older-aged study population (reflected by a mean BMI of 25.7 kg·m^{-2}). Obesity (BMI $\geqslant$30 kg·m^{-2}) was not chosen because of low sample size and its mechanical effects on lung function. Categorising BMI also enabled subgroup definitions in combination with ever-smoking.

We defined three susceptibility groups: never-smokers with high BMI, ever-smokers without, and ever-smokers with high BMI. Never-smokers without high BMI served as the reference group.

Statistical analysis

The distribution of the following was tabulated for the whole study population and susceptible groups: sex, age, educational level, smoking status, pack-years smoked, height, weight, BMI, FEV_1, FVC, FEV_1/FVC, $FEF_{25–75\%}$, pre-existing cardiopulmonary disease, bilirubin, C-reactive protein levels and genotypes of *UGT1A1* SNP rs6742078. To account for differential distribution of sex and age, adjusted bilirubin mean concentrations and standard errors were calculated for each group by predicting the values from a multiple linear regression model of log bilirubin on sex, age and group membership. Predictions were exponentiated to give units of μmol·L^{-1}.

Bilirubin values were transformed using the natural logarithm to achieve a more symmetric distribution. The cross-sectional association between log-transformed bilirubin values and FEV_1, FVC, FEV_1/FVC and $FEF_{25–75\%}$ from year 2002 was assessed using multiple linear regression adjusting stepwise for sex, age, height and weight, then educational level and study area, and finally ever-smoking status and pack-years. To check for interactions, dummy variables coding susceptibility group membership and multiplicative terms with log bilirubin were included into the models. Analyses were then stratified by susceptibility groups. Never-smoking participants without high BMI served as the reference group.

As an analysis of sensitivity, participants with elevated bilirubin levels (but normal liver enzymes) were excluded to assess whether the observed associations persisted in the normal range of serum bilirubin. Analyses were also repeated after excluding participants taking asthma medication (defined as a positive answer to the question ''Are you currently taking any medicines including inhalers, aerosols or tablets for asthma?''). Finally, analysis was stratified by the presence of cardiopulmonary disease to assess the role of pre-existing pathologies on the associations under study. Interaction was tested analogously with multiplicative interaction terms.

Mendelian randomisation analysis is based on the idea that during meiosis, the alleles of a genetic locus are passed on to the next generation by chance. If the genetic locus highly influences the levels of a biomarker, this natural randomised experiment allows inferences to be made on the causality of biomarker effects [25]. Accordingly, the association between rs6742078 genotypes and serum bilirubin was tested using Wilcoxon rank sum tests. Associations between rs6742078 genotypes and lung function were tested in regression models with the same covariates as for serum bilirubin analysis. Recessive models were analysed (allele TT

against GG/GT) to correspond with the functional impact of *UGT1A1* promoter $(TA)_7$ homozygosity [3]. In view of the small sample size (n=982) no stratified analysis was run.

All analyses were conducted using STATA version 10.1 (StataCorp, College Station, TX, USA). Adjusted mean bilirubin concentrations and standard errors were predicted using the "adjust" post-regression estimation command. Two-sided α-values of 0.05 and 0.1 were chosen as significance thresholds for main effects and interactions, respectively.

Results

Characteristics of the study population

Our study comprised 4195 subjects, of whom 1503 never-smokers without and 461 with high BMI, and 1713 ever-smokers without and 518 with high BMI. Overall, 53.2% were females, 22.1% were current smokers and 31.0% were former smokers (table 1). Average age was 51.9 years, and the mean FEV_1, FVC and $FEF_{25–75\%}$ were 3.2 L, 4.2 L, and 2.6 $L \cdot s^{-1}$, respectively. Median tobacco smoke exposure in ever-smokers was 17.0 pack-years (interquartile range 6–35). Median serum bilirubin in all participants was 7 $\mu mol \cdot L^{-1}$ (interquartile range 5–10 $\mu mol \cdot L^{-1}$). Across susceptibility groups, FEV_1/FVC and $FEF_{25–75\%}$ decreased with high BMI and smoking exposure. Groups with high BMI were older and less educated, while more males were in the smoking groups. Bilirubin serum values were apparently comparable across the groups, but after adjusting for differences in sex and age distribution, concentrations decreased progressively from 7.84 to 6.77 $\mu mol \cdot L^{-1}$ with increasing BMI and smoking exposure (online supplementary table S1).

Associations of bilirubin with lung function values

In the basic analysis models adjusting for sex, age, height and weight, FEV_1, FEV_1/FVC and $FEF_{25–75\%}$ were significantly and positively associated with serum bilirubin (table 2). One log unit increase in bilirubin, which corresponds to a 2.72-fold increase in serum concentration, was associated with increases as follows (β estimate (95% confidence interval)): FEV_1 36.4 (6.7–66.1) mL; FEV_1/FVC 0.9 (0.5–1.4)%; and $FEF_{25–75\%}$ 115.2 (58.5–172.0) $mL \cdot s^{-1}$. Effect estimates decreased after adjustment for tobacco smoke exposure, which also resulted in a loss of statistical significance for the FEV_1 association. In contrast, FEV_1/FVC and $FEF_{25–75\%}$ associations remained significant with estimated increases of 0.5 (0.1–1.0)% and 66.9 (11.4–122.5) $mL \cdot s^{-1}$ per log unit bilirubin. This corresponds to increases of 0.3 (0.1–0.7)% in FEV_1/FVC and 46.4 (7.9–84.9) $mL \cdot s^{-1}$ in $FEF_{25–75\%}$ over the observed interquartile exposure range (5–10 $\mu mol \cdot L^{-1}$ bilirubin, *i.e.* a doubling concentration).

For FEV_1/FVC, the interaction between log bilirubin and being an ever-smoker with or ever-smoker without high BMI was statistically significant ($p_{interaction}$ 0.007–0.023, data not shown). Interactions were also significant on $FEF_{25–75\%}$ ($p_{interaction}$ 0.012–0.086), except after pack-years adjustment ($p_{interaction}$ 0.111–0.163). Stratifying the analysis by susceptibility groups confirmed the interactions (table 3): no significant associations were present in never-smokers without high BMI. In never-smokers with high BMI, higher, although nonsignificant β estimates were observed for FEV_1, FEV_1/FVC and $FEF_{25–75\%}$. Associations between log bilirubin and FEV_1, FEV_1/FVC and $FEF_{25–75\%}$ were significant in ever-smokers without high BMI. They did not withstand pack-years adjustment for FEV_1 and FEV_1/FVC (21.7 (-23.6–67.0) mL and 0.6 (-0.1–1.2)% increase per log unit bilirubin, respectively), but only $FEF_{25–75\%}$ (88.7 (3.6–173.8) $mL \cdot s^{-1}$). The largest β estimates were observed in ever-smokers with high BMI across all FEV_1/FVC and $FEF_{25–75\%}$ models. After pack-years adjustment, each log unit bilirubin was significantly associated with 1.6 (0.1–3.2)% higher FEV_1/FVC and marginally associated with 167.6 (-23.0–358.3) $mL \cdot s^{-1}$ higher $FEF_{25–75\%}$. Over the observed interquartile range of bilirubin, this corresponds to increases of 1.1 (0.1–2.2)% FEV_1/FVC and 116.2 (-15.9–248.4) $mL \cdot s^{-1}$ $FEF_{25–75\%}$.

Sensitivity analyses

Association patterns were not substantially affected by the exclusion of participants with bilirubin levels above the normal range or those with asthma medication affected (fig. 2). Significant associations were observed in persons with and without pre-existing cardiopulmonary disease (online supplementary table S2). For FEV_1/FVC, estimates were significantly higher in the diseased group for education- and smoking-adjusted models ($p_{interaction}$ 0.086 and 0069, respectively; data not shown).

Associations of rs6742078 T-alleles with serum bilirubin and lung function values

Serum bilirubin values differed significantly according to the number of rs6742078 T-alleles (online supplementary table S3). Median concentrations were 6 and 7 $\mu mol \cdot L^{-1}$ for gentoypes GG and GT, *versus* 13 $\mu mol \cdot L^{-1}$ for homozygous T-alleles (p-value for Wilcoxon rank sum test <0.001).

Homozygous carriers of the rs6742078 T-allele had significantly higher levels of FEV_1/FVC and $FEF_{25–75\%}$ in progressively adjusted linear regression models (table 4), resulting in 1.7 (0.4–3.0)% higher FEV_1/FVC

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TABLE 1 Characteristics of the whole study sample and analysis subgroups

Variables	All[#]	Never-smokers		Ever-smokers	
		Without high BMI[¶]	With high BMI[+]	Without high BMI[§]	With high BMI[ƒ]
Female sex	2230 (53.2)	910 (60.5)	287 (62.3)	841 (49.1)	192 (37.1)
Age years	51.9±11.5	50.2±12.1	55.2±11.8	51.2±11.0	56.1±9.5
Education					
Low	274 (6.5)	70 (4.7)	63 (13.7)	86 (5.0)	55 (10.6)
Medium	2771 (66.1)	971 (64.6)	322 (69.8)	1143 (66.7)	335 (64.7)
High	1150 (27.4)	462 (30.7)	76 (16.5)	484 (28.3)	128 (24.7)
Body measures					
Height m	1.7±0.1	1.7±0.1	1.7±0.1	1.7±0.1	1.7±0.1
Weight kg	73.5±14.4	67.7±10.6	88.0±12.8	69.2±11.0	91.4±12.2
BMI kg·m^{-2}	25.7±4.3	23.8±2.6	31.9±3.5	24.0±2.6	31.7±3.1
Smoking history					
Current smoker	929 (22.1)	0 (0.0)	0 (0.0)	754 (44.0)	175 (33.8)
Pack-years[##]	17 (6.0–35.0)			15.6 (5.5–32.2)	22.4 (9.5–42.0)
Comorbidities					
Cardiopulmonary[¶¶]	605 (14.4)	173 (11.5)	83 (18.0)	243 (14.2)	106 (20.5)
Lung function					
FEV_1 L	3.2±0.8	3.2±0.8	2.9±0.9	3.2±0.8	3.0±0.8
FVC L	4.2±1.0	4.2±1.0	3.9±1.1	4.4±1.0	4.2±0.9
FEV_1/FVC %	74.7±7.5	76.2±6.9	74.7±7.0	74.0±7.6	72.8±8.1
$FEF_{25-75\%}$ L·s^{-1}	2.6±1.1	2.8±1.1	2.5±1.1	2.6±1.1	2.4±1.2
Biomarkers					
Bilirubin μmol·L^{-1}	7 (5–10)	7 (6–10)	7 (5–9)	7 (5–10)	7 (5–9)
C-reactive protein μmol·L^{-1}	1 (0.5–2.3)	0.8 (0.4–1.6)	1.9 (1.0–4.4)	0.9 (0.5–2.0)	2 (1.0–3.9)
Genotypes					
rs6742078					
GG	402 (40.9)	126 (36.7)	46 (41.1)	173 (43.0)	57 (45.6)
GT	453 (46.1)	171 (49.9)	50 (44.6)	180 (44.8)	52 (41.6)
TT	127 (12.9)	46 (13.4)	16 (14.3)	49 (12.2)	16 (12.8)

Data are presented as n, n (%), mean±SD or median (interquartile range). BMI: body mass index; FEV_1: forced expiratory volume in 1 s; FVC: forced vital capacity; $FEF_{25-75\%}$: forced expiratory flow at 25–75% of FVC. [#]: n=4195, with the exception of C-reative protein (n=4184) and genotypes (n=982); [¶]: n=1503, with the exception of C-reactive protein (n=1498) and genotypes (n=343); [+]: n=461, with the exception of genotypes (n=112); [§]: n=1713, with the exception of C-reactive protein (n=1707) and genotypes (n=402); [ƒ]: n=518, with the exception of genotypes (n=125); [##]: in ever-smokers only; [¶¶]: self-declared heart-disease, lung emphysema, chronic bronchitis or healthcare use for breathing problems in the last 12 months.

TABLE 2 Association of logarithmised bilirubin with lung function parameters in the whole study population

Outcome	Model covariates	β estimate (95% CI)	p-value
FEV_1 mL	Sex, age, height, weight	36.4 (6.7–66.1)	0.016
	All previous and education, study area	38.0 (8.4–67.6)	0.012
	All previous and ever-smoking, total pack-years	13.8 (-15.5–43.2)	0.356
FVC mL	Sex, age, height, weight	-6.4 (-41.4–28.7)	0.722
	All previous and education, study area	-4.9 (-39.6–29.7)	0.781
	All previous and ever-smoking, total pack-years	-14.5 (-49.3–20.4)	0.416
FEV_1/FVC %	Sex, age, height, weight	0.9 (0.5–1.4)	<0.001
	All previous and education, study area	1.0 (0.5–1.4)	<0.001
	All previous and ever-smoking, total pack-years	0.5 (0.1–1.0)	0.012
$FEF_{25-75\%}$ mL·s^{-1}	Sex, age, height, weight	115.2 (58.5–172.0)	<0.001
	All previous and education, study area	118.0 (61.9–174.1)	<0.001
	All previous and ever-smoking, total pack-years	66.9 (11.4–122.5)	0.018

Estimates are per natural log unit increase in bilirubin. Positive values mean higher lung function and negative values mean lower function. FEV_1: forced expiratory volume in 1 s; FVC: forced vital capacity; $FEF_{25-75\%}$: forced expiratory flow at 25–75% of FVC.

TABLE 3 Association of logarithmised bilirubin with lung function parameters stratified by ever-smoking and high body mass index (BMI)

Outcome for each model	Never-smokers				Ever-smokers			
	Without high BMI		With high BMI		Without high BMI		With high BMI	
	β (95% CI)	p-value	β (95% CI)	p-value	β (95% CI)	p-value	β (95% CI)	p-value
Subjects n	1503		461		1713		518	
FEV_1 mL								
Model 1	10.3 (-36.5–57.0)	0.667	14.9 (-76.2–106.0)	0.748	53.4 (7.6–99.2)	0.022	78.5 (-22.6–179.6)	0.128
Model 2	13.0 (-33.8–59.7)	0.586	22.4 (-68.1–112.8)	0.628	53.9 (7.9–99.8)	0.022	76.2 (-25.4–177.8)	0.142
Model 3					21.7 (-23.6–67.0)	0.348	43.0 (-57.9–144.0)	0.404
FVC mL								
Model 1	9.2 (-47.2–65.6)	0.749	-11.7 (-118.6–95.2)	0.830	4.0 (-50.4–58.5)	0.885	11.7 (-97.5–120.9)	0.834
Model 2	14.2 (-41.8–70.3)	0.619	-8.6 (-113.6–96.5)	0.873	5.7 (-48.4–59.7)	0.837	-1.4 (110.2–107.5)	0.980
Model 3					-7.9 (-62.3–46.6)	0.777	-26.8 (-135.9–82.3)	0.630
FEV_1/FVC %								
Model 1	0.1 (-0.6–0.7)	0.877	0.4 (-0.9–1.8)	0.514	1.1 (0.4–1.8)	0.001#	1.9 (0.3–3.4)	0.018#
Model 2	0.0 (-0.6–0.7)	0.928	0.6 (-0.7–1.9)	0.395	1.1 (0.4–1.8)	0.002#	2.0 (0.5–3.6)	0.008#
Model 3					0.6 (-0.1–1.2)	0.093#	1.6 (0.1–3.2)	0.033#
$FEF_{25–75}$ mL·s^{-1}								
Model 1	12.8 (-77.5–103.1)	0.781	45.5 (-129.0–220.0)	0.609	154.3 (66.8–241.7)	0.001#	202.3 (9.1–395.5)	0.040#
Model 2	12.7 (-76.9–102.2)	0.782	66.6 (-106.7–240.0)	0.451	151.1 (64.6–237.6)	0.001#	215.6 (25.1–406.0)	0.027#
Model 3					88.7 (3.6–173.8)	0.041	167.6 (-23.0–358.3)	0.085

Estimates are per natural log unit increase in bilirubin. Positive values mean higher lung function, negative mean lower function. FEV_1: forced expiratory volume in 1 s; FVC: forced vital capacity; $FEF_{25–75\%}$: forced expiratory flow at 25–75% of FVC. Model 1: adjusting for sex, age, height, weight; model 2: adjusting additionally for education and study area; model 3: adjusting additionally for pack-years smoked. #: statistically significant difference compared to never-smokers without high BMI (p-value for interaction <0.1).

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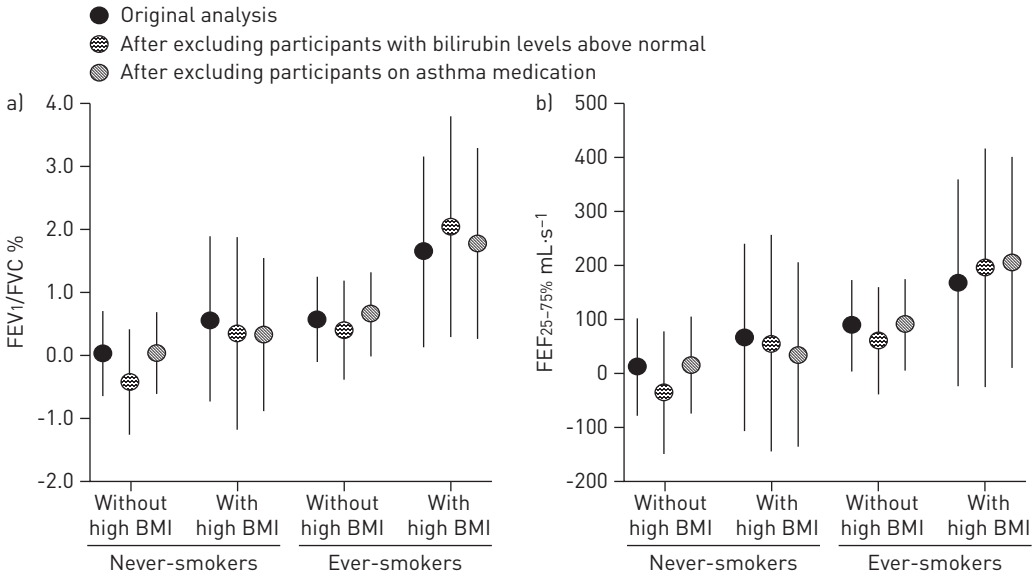

FIGURE 2 Sensitivity analysis of bilirubin associations with a) forced expiratory volume in 1 s (FEV_1)/forced vital capacity (FVC) and b) forced expiratory flow at 25–75% of FVC ($FEF_{25–75\%}$). Estimates are indicated per log unit increase in bilirubin. Models adjusted for sex, age, height, weight, education, study area and, in ever-smoker strata, additionally for pack-years smoked. BMI: body mass index.

and 192.5 (30.8–354.2) $mL \cdot s^{-1}$ higher $FEF_{25–75\%}$ than GT/GG carriers after pack-years adjustment. No significant associations were observed for FEV_1 and FVC.

Discussion

In the population-based SAPALDIA study, we found significant positive associations between serum levels of bilirubin and lung function parameters FEV_1/FVC and $FEF_{25–75\%}$ after adjusting for the effects of sex, age, education, height and weight, as well as tobacco smoke exposure. When stratifying the study population according to states of increased susceptibility, associations persisted only in participants with

TABLE 4 Associations of rs6742078 genotypes with lung function

Outcome for each model	Estimates for recessive effects of rs6742078 (allele TT *versus* GG/GT)[#]	
	β (95% CI)	p-value
FEV_1		
Model 1	27.7 (-65.9–121.2)	0.562
Model 2	40.6 (-52.6–133.8)	0.394
Model 3	34.7 (-57.2–126.7)	0.459
FVC		
Model 1	-58.4 (-162.0–45.2)	0.269
Model 2	-42.8 (-145.8–60.2)	0.415
Model 3	-44.3 (-147.3–58.7)	0.399
FEV_1/FVC		
Model 1	1.7 (0.4–3.1)	0.013
Model 2	1.8 (0.4–3.2)	0.010
Model 3	1.7 (0.4–3.0)	0.013
$FEF_{25–75\%}$		
Model 1	190.7 (22.9–358.4)	0.026
Model 2	205.6 (40.6–370.7)	0.015
Model 3	192.5 (30.8–354.2)	0.020

[#]: rs6742078 was coded as recessive effect to correspond with functional effects of the *UGT1A1* promoter polymorphism: (TA)7/7 *versus* (TA)6/6 and (TA)6/7. Genetic data was available for n=982 participants. FEV_1: forced expiratory volume in 1 s; FVC: forced vital capacity; $FEF_{25–75\%}$: forced expiratory flow at 25–75% of FVC; model 1: adjusting for sex, age, height and weight; model 2: additionally adjusting for education and study area; model 3: additionally adjusting for ever-smoking and pack-years smoked.

high BMI and were increased and significant in ever-smoking strata. Our results were robust to sensitivity analysis, and consistent associations were observed for genetically elevated bilirubin (due to rs6742078).

These findings are coherent with previous epidemiological evidence showing the beneficial effects of elevated serum bilirubin on respiratory outcomes [5–7]. These findings suggest beneficial effects on lung function, but refine their scope to persons with subclinical inflammation and oxidative stress exposure (or cardiopulmonary disease). The significant associations with $FEF_{25–75\%}$ and FEV_1/FVC might point to an inverse relationship with small airway obstruction. Due to their large surface area, small airways are an important compartment of chronic respiratory disease development [26, 27], where subclinical changes often manifest first, and accordingly, strongest associations presented at this site in our general population sample. Smoking adjustment decreased association estimates considerably, which was expected given its known inverse relationship with lung function and serum bilirubin, resulting in positive confounding of the bilirubin lung function association.

We can currently only speculate about the mechanisms by which serum bilirubin might influence lung function. Intracellular mechanisms may be more important in the lung than the known effects in serum and blood vessels, as bilirubin penetrates cell walls at physiological pH values [28]. Besides scavenging oxidants, bilirubin inhibits membrane-bound nicotinamide adenine dinucleotide phosphate-oxidase, one of the major intracellular sources of reactive oxygen species [29]. Furthermore, bilirubin infusions have been shown to downregulate inflammation in murine lung injury models [30].

Because of the cross-sectional design, the observed associations cannot be interpreted clearly regarding the temporal relationship of outcome and exposure: the findings could arise from a protective effect of pre-existing elevated serum bilirubin, preventing tissue damage by inflammatory and oxidative stress reactions, or bilirubin levels could represent a reactive marker of the individual oxidative stress burden and ensuing bilirubin consumption. Our results rather suggest a causal association for different reasons. First, the observed associations between serum bilirubin levels and lung function were consistently replicated in participants with genetically elevated serum bilirubin. Secondly, associations grew stronger across increasing states of subclinical inflammation and oxidative stress (*i.e.* in persons with high BMI, ever-smokers, or both), and even persisted in persons with cardiopulmonary disease. This would probably not be the case for a reactive biomarker. Finally, we observed similar but less strong associations in the normal range of serum bilirubin.

Our study has few limitations besides its cross-sectional design. Genotyping data was only available in a subset of the population. Our data was potentially affected by measurement error, as lung function was tested only twice, but the resulting misclassification would be random and primarily affect study power. In contrast, our study has several strengths. The population-based design allowed investigation of lung function as a pre-clinical marker of disease processes. The large study sample and detailed data on blood markers, body size and lifestyle factors allowed stratification of the analysis to study associations across subclinical conditions of inflammation and oxidative stress. This offered new insights into the possible health-related effects of bilirubin. Our large study sample also meant that different sensitivity analyses could be conducted. Finally, our study benefited from a standardised spirometry protocol and adherence to strict quality control criteria.

In conclusion, we found significant positive associations of serum bilirubin levels with lung function in persons with increased inflammation and oxidative stress. From a public health perspective, it would be important to clarify whether the observed relationship is causal using prospective studies that include genetic information. Bilirubin could have large preventive and therapeutic potential, given the prevalence of smoking and high BMI in Western populations.

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