ELSEVIER

Atherosclerosis 160 (2002) 449–456

ATHEROSCLEROSIS

www.elsevier.com/locate/atherosclerosis

Gilbert syndrome and ischemic heart disease: a protective effect of elevated bilirubin levels☆

Libor Vítek [a,d,*], Milan Jirsa Jr. [b], Marie Brodanová [b], Milan Kaláb [b], Zdeněk Mareček [b], Vilém Danzig [b], Ladislav Novotný [c], Petr Kotal [d]

[a] *4th Department of Internal Medicine, 1st Medical Faculty, Charles University Prague, U Nemocnice 2, 128 08 Prague, Czech Republic*
[b] *1st Department of Internal Medicine, 1st Medical Faculty, Charles University Prague, Prague, Czech Republic*
[c] *3rd Department of Internal Medicine, 1st Medical Faculty, Charles University Prague, Prague, Czech Republic*
[d] *Institute of Clinical Biochemistry, 1st Medical Faculty, Charles University Prague, Prague, Czech Republic*

Received 21 September 2000; received in revised form 26 June 2001; accepted 29 June 2001

Abstract

Background: Oxidation processes play an important role in atherogenesis. Bilirubin IXα is recognised as a potent antioxidant. In the present study, we assessed the role of elevated serum bilirubin levels in the prevention of ischemic heart disease (IHD). *Methods:* The occurrence of IHD was determined in Gilbert syndrome (GS) patients above 40 years ($n = 50$). The diagnosis was based on past medical history and ECG criteria. The occurrence was related to that of the comparable general population ($n = 2296$). Serum biochemistry, including the total antioxidant status was evaluated in the GS subjects, IHD patients ($n = 38$) and control subjects ($n = 38$). *Results:* The prevalence of IHD in GS subjects (aged 49.7 ± 9.0 years) was 2% (0.05–10.7%, 95% confidence interval), compared to 12.1% in a general population ($P < 0.05$). Bilirubin, total antioxidant capacity and high density lipoprotein (HDL) cholesterol were found to be significantly higher in GS subjects compared to control groups ($P < 0.05$). According to linear discriminant analysis, hyperbilirubinemia rather than elevation of HDL cholesterol levels seemed to be more important in protection from IHD. *Conclusions:* In the present study, low prevalence of IHD in GS subjects was detected. It may be presumed that chronic hyperbilirubinemia prevent the development of IHD by increasing the serum antioxidant capacity.

Keywords: Bilirubin; Gilbert syndrome; Oxidation; Ischemic heart disease

1. Introduction

Cardiovascular disease is the most common cause of death and is responsible for 50% of all mortality in developed countries [1]. A large number of risk factors have been described to be involved in the process of atherogenesis [1]. Among them, elevated levels of low density lipoprotein (LDL) cholesterol is the most important and its oxidative modification in the process of atherogenesis seems to be crucial [2]. LDL can be oxidatively modified by transition metals and by all major cells of the arterial wall [3]. Since oxidative modification of LDL results from lipid peroxidation, water- and lipid-soluble antioxidants should have a prominent effect in preventing this modification. This suggestion has been confirmed in numerous studies with a variety of natural or synthetic antioxidants such as vitamins A, C and E [4], probucol, phenothiazines, calcium antagonists, agents complexing copper and iron ions, and others [5].

Free radicals occur ubiquitously in the body and can disrupt the function of many cells and molecules. Natural antioxidant defences have evolved to protect humans against deleterious effects of free radicals. The primary enzymatic defences are intracellular, but other

Abbreviations: CI, confidence interval; GS, Gilbert syndrome; HDL, high density lipoprotein; HO, heme-oxygenase; IHD, ischemic heart disease; LDL, low density lipoprotein; SRBI, scavenger receptor BI; TAS, total antioxidant status; UCB, unconjugated bilirubin.

☆ Part of this study was presented in a preliminary form at the 33rd Annual Meeting of the European Association for the study of the liver in Lisboa, Portugal, 1998.

* Corresponding author. Fax: +42-02-2492354.
E-mail address: vitek@cesnet.cz (L. Vítek).

0021-9150/02/$ - see front matter
PII: S0021-9150(01)00601-3

antioxidant defences are largely extracellular, including antioxidative substrates such as transferrin, ceruloplasmin, albumin, uric acid [6,7], and unconjugated bilirubin (UCB), the predominant bile pigment in the intravascular compartment [8]. Its formation is mediated by heme-oxygenase (HO), the rate-limiting enzyme of the heme catabolism. Three distinct isoforms of HO have been identified; one is inducible (HO-1) belonging to the family of heat-shock protein [8], whereas the other forms are constitutive (HO-2 [9], and HO-3 [10]). The inducible form of HO is affected by numerous factors including oxidised hemoglobin, heavy metal ions, hydrogen peroxide, ultraviolet A radiation, nitric oxide, and free oxygen radicals [9,11]. In this respect, bilirubin seems to represent an important endogenous agent with cytoprotective activity against oxidative stress, because of its potent antioxidant properties which were demonstrated in in vitro [8,12–16], animal [17,18] and human studies [19–25]. In addition to being a potent antioxidant, bilirubin is considered to play a role in tissue protection against inflammatory damage by its anticomplement action [26]. It has been reported that albumin, which appears in inflammatory exudate, carries bilirubin across the vascular wall into the sites of potential oxyradical damage by phagocytic cells [27]. Furthermore, bilirubin may reduce oxidative stress induced by hypertension [28].

Three major clinical studies regarding the possible influence of bilirubin levels on ischemic heart disease (IHD) have been published. In 1994, Schwertner et al. [21] described an inverse relationship between serum bilirubin concentrations and the risk of coronary artery disease. Breimer et al. [22] demonstrated in a prospective study of 7685 middle-aged British men an enhanced risk of IHD in patients with low bilirubin levels, although a similar tendency was also observed in the hyperbilirubinemic subjects, making the relationship U-shaped. In another study done by Hopkins et al. [23] significantly lower bilirubin levels in patients with coronary artery disease as compared to control subjects were documented. The relevance of serum bilirubin as a risk factor inversely related to the coronary artery disease was suggested also in other studies [24,25].

In our study, we focused on the occurrence of IHD in a group of patients with confirmed diagnosis of Gilbert syndrome (GS) characterised by sustained unconjugated hyperbilirubinemia. GS is due to a partial deficiency of bilirubin UDP-glucuronosyltransferase in the liver tissue with $\approx 6\%$ prevalence [29]. Mild unconjugated hyperbilirubinemia in the range of 20–100 μmol/l represents a typical clinical picture.

2. Materials and methods

Five groups of subjects were studied. Informed consent was obtained from all of the subjects prior to enrolment. The study was approved by the Ethics Committee of the Teaching Hospital, 1st Medical Faculty, Charles University, Prague.

A group of 50 patients with GS (Group A, consisting of 35 men and 15 women) was enrolled from the Hepatology Outpatient Medical Centre, Faculty Hospital of the Charles University in Prague. The patients were selected on the basis of chronic unconjugated hyperbilirubinemia in the absence of any hemolytic disease and/or any other hepatic function alteration; the second criterion was an age of 40 years or more. A diagnostic liver biopsy was performed in six patients in whom laboratory findings at the onset of follow-up were not completely diagnostic for GS; normal histology was found in all cases. At least five determinations of serum bilirubin concentration were performed in every patient within the last 3 years prior enrolment. Serum bilirubin levels were within the range of 20–70 μmol/l. The diagnosis of IHD was based on past medical history of symptomatic IHD (occurrence of stenocardia, positive effect of nitrate therapy) and ECG criteria (specific changes of ST segment, presence of Q wave, incidental left bundle branch block). When screening for IHD, identical criteria were applied to all the subjects of the screened groups. In one patient with suspect IHD cyclo-ergometry, selective coronary arteriography, carotid ultrasonography and dobutamine stress echocardiography were performed to assess the extent and significance of possible atherosclerosis. During the 3-year follow-up of IHD 12 patients were lost from the study due to personal reasons.

Groups B and C were used to compare biochemical parameters of Group A subjects (Table 1). Group B

Table 1
Characteristics of groups involved in the study

Group	Number of subjects	Sex distribution (male/female)	Age (years, mean ± S.D.)
GS (A)	50	35/15	49.7 ± 9.0
IHD (B)	38	33/5	50.5 ± 7.2
Controls (C)	38	14/24	47.3 ± 4.6
Controls (D)	2296	1520/776	50.5 ± 6.3
Controls (E)	316	184/132	51.8 ± 7.6

GS, Gilbert syndrome patients; IHD, patients with ischemic heart disease; Controls, control groups.

consisted of 38 patients with an apparent IHD (33 men and 5 women), 40–60 years old. These patients were consecutively seen, and consisted either of those with a confirmed diagnosis of an acute myocardial infarction, or a severe unstable angina pectoris requiring intravenous nitrate therapy. A small part ($n = 7$) consisted of patients admitted for a selective coronarography.

Group C consisted of 38 consecutive healthy blood donors (14 men and 24 women) of over 40 years recruited from the blood transfusion unit. These subjects were selected on the basis of the absence of any major disease at the time of enrolment to the blood donation program and, therefore, this group does not represent a sample of a general population. Groups B and C individuals were subjected to the same tests as the population with GS. In Group C, neither symptoms of IHD nor ECG findings indicative of IHD (as defined above) were observed. All the electrocardiograms were assessed by the same cardiologist.

Serum lipid profile, total antioxidant status (TAS) and bilirubin concentrations were determined on automatic analyser (model 717; Hitachi, Tokyo, Japan). Total cholesterol was measured enzymatically by the cholesterol oxidase assay and high density lipoprotein (HDL) cholesterol was measured by the same procedure after precipitation of LDL and VLDL with magnesium sulphate and phosphotungstic acid. Bilirubin was analysed by the diazo reaction according to Jendrassik and Gróf.

The TAS was determined spectrophotometrically using kit TAS, Randox Laboratories Ltd., Antrim, UK. In this assay 2,2′-azino-di-[3-ethylbenzthiazoline sulphonate] (ABTS®) is incubated with a peroxidase (metmyoglobin) and H_2O_2 to produce the radical cation ABTS®*. This has a relatively stable blue-green colour, which is measured kinetically at 600 nm. Antioxidants in the added sample cause suppression of the colour production to a degree, which is proportional to their concentration.

The influence of UCB (Sigma, St. Louis, MO) artificially added to the serum on the TAS was determined as follows. UCB was dissolved in 0.1 M NaOH to get the final concentration of 1 mmol/l. Appropriate amounts of the solution were added to the standard serum (Randox Laboratories Ltd.) in order to increase bilirubin concentration by 6.25, 12.5, 25, 50 and 100 μmol/l.

Group D (Table 1) was used to assess the prevalence of manifesting IHD in a general population in order to compare it with that of GS patients. This group comprised 2296 subjects derived from the outpatient clinic taking care of employees of a large administrative institution in Prague. These individuals were selected according to age and sex criteria to meet population characteristics of GS patients. The presence of IHD was assessed according to the same criteria, i.e. symptoms or ECG findings indicative of IHD, as defined above.

Group E (Table 1), comprising 316 healthy subjects with comparable age and sex distribution to that of GS group, was used to compare predicted probability of IHD based on the evaluation of multiple standard risk factors for IHD between these two groups (see below). These subjects were recruited from a pending population study focused on the evaluation of risk factors for IHD.

2.1. Statistical analysis

The one way analysis of variance (ANOVA) was used to compare means of measured variables in examined Groups A–C. In case of statistically significant difference at the 5% level, the Scheffe method of multiple comparisons was used to reveal which difference between compared groups was responsible for this result.

The mean bilirubin concentrations were compared after logarithmic transformation in order to meet requirements for ANOVA application and fulfil the equality of variances assumption. A Kolmogorov–Smirnov test was performed on the logarithm of bilirubin concentration for all groups separately to check possible departure from normality; all these results were non-significant.

Fisher linear discriminant function based on bilirubin and HDL cholesterol levels was performed to discriminate between the groups of GS and IHD [30].

The calculation of the 95% confidence interval (CI) was used in order to determine the predicted prevalence in GS population. The prevalence of IHD was seen as a binomial distribution parameter (n, p, where $n = 50$ and $p = 1/50$).

Comparison of proportions using χ^2 test was carried out to compare the prevalence of IHD in Groups A and D.

Probability equations [31] based on the evaluation of multiple standard risk factors for IHD (including age, sex, systolic blood pressure, total cholesterol, HDL cholesterol, smoking, diabetes mellitus and ECG signs for left ventricular hypertrophy) were used to predict probability of IHD in Groups A and E.

Poisson distribution probability test was performed to compare observed and predicted incidence of IHD among patients with GS.

3. Results

The mean age of subjects in examined groups was comparable (Table 1) ($P > 0.05$). One out of 50 examined patients with GS was found to suffer from symptomatic IHD. The patient was 60-year-old female, her IHD was diagnosed at the age of 57 years by the appearance of exercise-induced angina pectoris. She

Table 2
Serum bilirubin and total antioxidant status in Gilbert syndrome, ischemic heart disease patients and control subjects (mean ± S.D.)

Group	Bilirubin* (μmol/l)	TAS* (mmol/l)
GS	32.6 ± 13.5	1.433 ± 0.14
IHD	9.0 ± 2.7	1.296 ± 0.14
Controls	9.1 ± 2.7	1.323 ± 0.13

Total, total antioxidant status; GS, Gilbert syndrome patients; IHD, patients with ischemic heart disease; Controls, control healthy blood donors.
* GS vs. IHD and controls: $P<0.05$.

had a positive cyclo-ergometry test, but selective coronary arteriography did not reveal any coronary stenosis. It has been concluded that she had ischemic disease of small coronary vessels. Dobutamine stress echocardiography did not reveal any pathological finding, and carotid ultrasonography with a measurement of intimal thickness was completely within normal range. No apparent risk factors for IHD were found to be present in this patient. In all remaining hyperbilirubinemic subjects symptoms of IHD were absent.

On the basis of this finding the prevalence of IHD in the population of GS patients was predicted to range between 0.05 and 10.7% (95% CI). In comparison, the prevalence of symptomatic IHD in a general unselected population of comparable age and sex distribution was found to reach 12.1% ($P<0.05$). During the 3-year follow-up, no patient with GS (Group A) developed symptomatic IHD. Based on multivariable analysis of standard risk factors (see above) [31] the predicted 3-year incidence of IHD in patients with GS (Group A) was 3.1% for males and 0.5% for females. This was comparable to the predicted incidence of IHD in healthy comparable population (Group E; 3.2% for males, 1.1% for females). Poisson distribution probability test of observed and predicted 3-year incidence of IHD in male GS subjects (0 vs. 3.1%) revealed statistically significant difference between compared parameters ($P<0.05$). Due to a limited number of patients, this test could not be used in females.

The standard biochemical parameters were compared among Groups A, B and C in order to find out a factor responsible for beneficial effect on the prevention of development of IHD. As expected, significantly higher serum bilirubin levels were found in the group of GS patients as compared to patients with IHD and control subjects ($P<0.05$; Table 2). Similarly, a significant difference in the TAS was detected between the group of GS patients, and IHD patients and control subjects ($P<0.05$; Table 2). No significant difference was found between the groups of IHD patients and control subjects. The total and LDL cholesterol concentrations did not differ significantly among all three groups ($P>0.05$; Table 3). However, the concentration of HDL cholesterol was significantly different in ischemic patients as compared to a hyperbilirubinemic group ($P<0.05$; Table 3).

Other risk factors for IHD evaluated in GS patients such as body mass index, alcohol consumption and physical activity, were either within a normal range or did not differ from the general population. Prevalence of smoking was significantly lower in GS subjects as compared to the general population (4 vs. 24%, $P<0.05$). However, low prevalence of smoking in GS subjects did not significantly influence predicted probability of IHD, as proved by analysis of multiple standard risk factors (see above). Moderate arterial hypertension was diagnosed in one GS patient, all other patients were completely healthy. Linear discriminant analysis used as a technique to assess the preventive role of UCB and HDL levels on the development of IHD revealed a positive correlation between elevated bilirubin levels and a lower incidence of IHD. Linear discriminant function for logarithms of values of bilirubin and HDL cholesterol concentrations separated the patients with GS from those with IHD quite well (Fig. 1). The reclassification results of the same data showed only two misclassified observations of the estimated linear discriminant functions.

The effect of UCB artificially added to the serum was evaluated with respect to the differences in bilirubin levels between GS patients and control subjects. An addition of UCB to the serum with a defined total antioxidant capacity led to an elevation of this parameter from 1.37 to 1.76 mmol/l ($P<0.05$) in response to a progressive increase of the bilirubin concentration (Table 4).

Table 3
Lipid profile in Gilbert syndrome and ischemic heart disease patients, and control subjects (mean ± S.D.)

Group	Total cholesterol* (mmol/l)	LDL cholesterol* (mmol/l)	HDL cholesterol** (mmol/l)
GS	5.6 ± 0.9	3.5 ± 0.9	1.5 ± 0.5
IHD	5.9 ± 1.5	3.7 ± 1.0	1.1 ± 0.2
Controls	5.2 ± 0.8	3.3 ± 0.6	1.3 ± 0.3

GS, Gilbert syndrome patients; IHD, patients with ischemic heart disease; Controls, control healthy blood donors.
* GS vs. IHD and controls: $P>0.05$; ** GS vs. IHD: $P<0.05$.

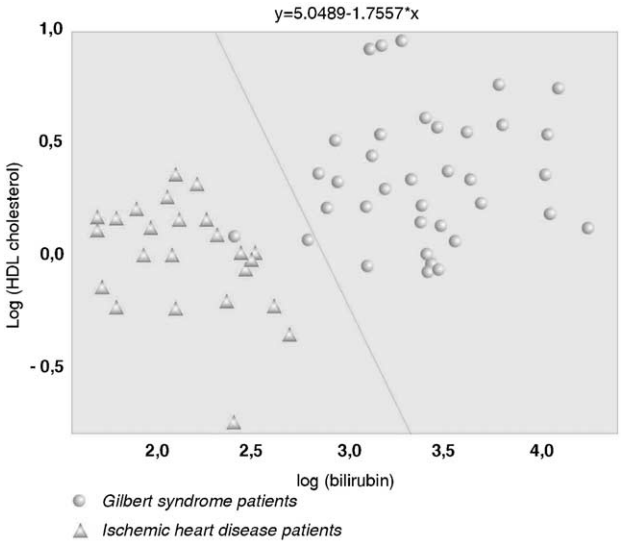

Fig. 1. Linear discriminant analysis of logarithmic values of serum bilirubin and HDL cholesterol levels.

4. Discussion

IHD is the most common cause of morbidity and mortality in developed countries [1]. The prevalence of symptomatic IHD in a 40–60-year-old Slavonic population ranges between 10 and 20%, as was shown in previous epidemiological studies from Czech Republic, Poland and Russia [32–34]. These data are consistent with our findings showing 12.1% prevalence of symptomatic IHD among 40–60-year-old subjects representative of the general population (Group D).

In the present study, we describe a low cross-sectional prevalence of IHD in the subset of patients with GS. Despite the limited number of patients with GS, highest predicted prevalence (95% CI) of IHD in a population of GS subjects is significantly lower than that of the general population (10.7 vs. 12.1%, $P < 0.05$). Moreover, the patient with diagnosed IHD has suffered from a mild form of the disease, as evidenced by the clinical picture as well as normal findings of selective coronary arteriography. Also, results of dobutamine stress echocardiography, used as a tool for prediction of the prognosis of IHD in patients with suspect atherosclerosis of small coronary vessels [35], and ultrasonography of carotid arteries with measurement of intimal thickness were completely, physiological, suggesting perhaps a mild form of atherosclerosis in our patient.

Table 4
The influence of addition of unconjugated bilirubin to the serum with defined TAS activity

Added UCB (μmol/l)	Total bilirubin (μmol/l)	TAS (mmol/l)
0[a]	24.5	1.368
6.25	31.9	1.418
12.50	40.6	1.462
25.00	55.8	1.505
50.00	79.6	1.570
100.00	133.0	1.764

TAS, total antioxidant status.
[a] Serum with defined TAS capacity.

During a 3-year follow-up no GS patient out of 38 followed developed symptoms or ECG changes indicative of IHD. Although the size of the GS population is not very large, the difference between observed and predicted 3-year incidence of IHD reached statistical significance ($P < 0.05$).

Elevated bilirubin and HDL cholesterol levels were the only different standard serum parameters among subjects with GS, those with IHD, and controls. Considering potent antioxidant effects reported previously [19–25], elevated concentrations of UCB may indeed serve as a protective factor in the development of IHD. This conclusion is further substantiated by the results of the linear discriminant analysis, suggesting that elevated serum bilirubin levels may play an even more important role in the prevention of development of IHD than an elevation of serum HDL cholesterol concentrations (Fig. 1). In addition, the predicted probability of IHD based on standard risk factors (including HDL cholesterol and smoking) was comparable between male GS subjects and control Group E male subjects. This suggests that neither elevated HDL cholesterol nor low smoking prevalence, but other factors not used in the equation (such as bilirubin as the only other different standard serum parameter) must be responsible for lower 3-year incidence of IHD observed in male GS subjects. In fact, an inverse relationship between dietary intake of antioxidant substances such as vitamins A, C, and E and the risk of IHD was shown in several studies [36–39], indicating that plasma antioxidants are essential for prevention of IHD.

Interestingly, compared to IHD patients HDL cholesterol was elevated only in GS patients and not in a control group of healthy blood donors. It has been demonstrated that the majority of oxidised lipids in human plasma are associated with HDL [40]. Because of that, it is tempting to speculate whether elevated bilirubin levels might protect HDL apoproteins and lipids from being oxidised, thus reducing the catabolic rate mediated by HDL receptor SRBI [41].

A number of assays have been introduced to determine the antioxidant activity of body fluids [42]. Measurement of TAS by ferrylmyoglobin-ABTS assay represents one of the common method [42]. In our study, TAS was significantly higher in patients with GS as compared with that of patients with IHD and control subjects ($P < 0.05$). Bilirubin was the only enhanced standard antioxidant parameter in examined

sera (including albumin, ceruloplasmin, transferrin and uric acid, data not shown). No significant difference in TAS (and also in serum bilirubin and HDL cholesterol) was found between the groups of patients with IHD and control subjects indicating involvement of other contributing factors such as arterial hypertension, diabetes mellitus, obesity, smoking and possible genetic or other factors in the atherogenesis of our patients with IHD. It is apparent that differences in these parameters must be present to account for the absence of IHD in our subset of blood donors. The progressive increase of TAS was related to the increase of bilirubin concentration, as was proved in the in vitro assay (Table 4). This finding is consistent with an increased plasma antioxidant capacity found in jaundiced new-born infants [43] and patients with hyperbilirubinemia due to sickle cell disease [44].

Up to now the effects of bilirubin on development of IHD have been studied only under conditions of physiological concentrations of bilirubin [19–21,23–25]. In only one study [22], a subset of hyperbilirubinemic subjects was examined and these patients were not protected from development of IHD. However, this subgroup was not well defined and involved also subjects with raised liver enzymes. Correction of this subgroup only to hyperbilirubinemic patients without elevated liver enzyme reveals that these subjects had surprisingly the lowest incidence rate of IHD among all the other groups examined [22]. This fact, although not noted by the authors, is consistent with our results.

According to our data the protective antiatherogenic effect rises with an increase of the serum bilirubin level. This is supported by Dennery et al. [18], who reported reduced oxidative injury in neonatal hyperbilirubinemic rats, and also by Benaron et al. [20], who observed significantly lower incidence of neonatal complications in hyperbilirubinemic infants. An evidence of potential antiatherogenic effects of elevated bilirubin levels was also added in the recent clinical studies [21–23]. These findings are consistent with experimental animal data showing protective effects of hyperbilirubinemia induced by up-regulation of HO-1 on postischemic myocardial dysfunction [45]. HO-1 was also reported to be induced in heart by hemodynamic stress such as from right-sided heart failure [46], heart and renal ischemia and reperfusion [47,48], and hypertension [49]. Furthermore, HO-1 overexpression in mouse cardiac allografts was shown to protect from transplant atherosclerosis due to possible anticomplement and antioxidant effects of bilirubin [50]. This is consistent with significant elevation of UCB levels described in patients with acute myocardial infarction [51], suggesting that under in vivo conditions HO up-regulation may indeed be implicated in the protection against oxidative stress. These data indicate that bilirubin generated via HO-1 up-regulation may have a cytoprotective role in the cardiovascular system and these effects may account for a low incidence of IHD in hyperbilirubinemic subjects observed in this study.

In conclusion, significantly lower occurrence of IHD in hyperbilirubinemic subjects is reported. It seems that elevated levels of serum bilirubin may play an important role in the prevention of IHD by inhibition of deleterious effects of oxidative stress. It is possible that the beneficiary effect of UCB on the prevention of IHD may be additive or synergistic to that of HDL cholesterol. In fact, low serum bilirubin levels have recently been proposed as a factor predicting severity of coronary artery disease independently of HDL cholesterol concentrations [52].

Acknowledgements

This work was supported by grants no. 2226-3 and 4866-3 given by the Czech Ministry of Health, and a grant no. J 13/98:111100002 given by the Czech Ministry of Education. Authors thank Ivana Grohová, MD for her help with the examination of healthy blood donors, and Monika Pacltová, MSc. for the determination of the total antioxidant status. Authors also thank Professor Martin C. Carey for a critical reading of the manuscript.

References

[1] Ross R. The pathogenesis of atherosclerosis: a perspective for the 1990s. Nature 1993;362:801–9.
[2] Aviram M. Modified forms of low density lipoprotein and atherosclerosis. Atherosclerosis 1993;98:1–9.
[3] van Hinsbergh VWM, Scheffer M, Havekes L, Kempen HJM. Role of endothelial cells and their products in the modification of low-density lipoproteins. Biochim Biophys Acta 1986;878:49–64.
[4] Gibaldi M. Antioxidant vitamins and health. J Clin Pharmacol 1996;36:1093–9.
[5] Esterbauer H, Gebicki J, Puhl H, Jürgens G. The role of lipid peroxidation and antioxidants in oxidative modification of LDL. Free Rad Biol Med 1992;13:341–90.
[6] Gutteridge JMC. Antioxidant properties of the proteins caeruloplasmin, albumin and transferrin. A study of their activity in serum and synovial fluid from patients with rheumatoid arthritis. Biochim Biophys Acta 1986;869:119–27.
[7] Becker B. Towards the physiological function of uric acid. Free Rad Biol 1993;14:615–31.
[8] Stocker R, Yamamoto Y, McDonagh A, Glazer AN, Ames NB. Bilirubin is an antioxidant of possible physiological importance. Science 1987;235:1043–6.
[9] Maines MD. The heme oxygenase system: a regulator of second messenger gases. Ann Rev Pharmacol Toxicol 1997;37:517–54.
[10] McCoubrey WK Jr, Huang TJ, Maines MD. Isolation and characterization of cDNA from the rat brain that encodes hemoprotein heme oxygenase-3. Eur J Biochem 1997;247:725–32.
[11] Motterlini R, Foresti R, Intaglietta M, Winslow RM. NO-mediated activation of heme-oxygenase: endogenous cytoprotection

against oxidative stress to endothelium. Am J Physiol Heart Circ Physiol 1996;270:H107–14.
[12] Neužil J, Stocker R. Free and albumin-bound bilirubin are efficient co-antioxidants for α-tocoferol, inhibiting plasma and low density lipoprotein lipid peroxidation. J Biol Chem 1994;269:16712–9.
[13] Hulea SA, Wasowitz E, Kummerow FA. Inhibition of metal catalyzed oxidation of low-density lipoprotein by free and albumin bound bilirubin. Biochim Biophys Acta 1995;1259:29–38.
[14] Wu TW, Fung KP, Wu J, Yang CC, Weisel RD. Antioxidation of human low density lipoprotein by unconjugated and conjugated bilirubins. Biochem Pharmacol 1996;5l:859–62.
[15] Wu TW, Fung KP, Yang CC. Unconjugated bilirubin inhibits the oxidation of human low density lipoprotein better than Trolox. Life Sci 1994;54:477–81.
[16] Minetti M, Mallozzi C, DiStasi AMM, Pietroforte D. Bilirubin is an effective antioxidant of peroxynitrite mediated protein oxidation in human blood plasma. Arch Biochem Biophys 1998;352:167–74.
[17] Yamaguchi T, Terakado M, Horio F, Aoki K, Tanaka M, Nakajima H. Role of bilirubin as an antioxidant in an ischemia-reperfusion of rat liver and induction of heme oxygenase. Biochem Biophys Res Commun 1996;223:129–35.
[18] Dennery PA, McDonagh AF, Spitz DR, Rodgers PA, Stevenson DK. Hyperbilirubinemia results in reduced oxidative injury in neonatal Gunn rats exposed to hyperoxia. Free Rad Biol Med 1995;19:395–404.
[19] Gopinathan V, Miller JN, Milner AD, Rice-Evans CA. Bilirubin and ascorbate antioxidant activity in neonatal plasma. FEBS Lett 1994;349:197–200.
[20] Benaron DA, Bowen FW. Variation of initial serum bilirubin rise in newborn infants with type of illness. Lancet 1991;338:78–81.
[21] Schwertner HA, Jackson WG, Tolan G. Association of low serum concentration of bilirubin with increased risk of coronary artery disease. Clin Chem 1994;40:18–23.
[22] Breimer LH, Wannamethee G, Ebrahim S, Shaper AG. Serum bilirubin and risk of ischemic heart disease in middle-aged British men. Clin Chem 1995;41:1504–8.
[23] Hopkins PN, Wu LL, Hunt SC, James BC, Vincent MC, Williams RR. Higher serum bilirubin is associated with decreased risk for early familial coronary artery disease. Arteroscler Thromb Vasc Biol 1996;16:250–5.
[24] Levinson SS. Relationship between bilirubin, apolipoprotein B, and coronary artery disease. Ann Clin Lab Sci 1997;27:185–92.
[25] Madhavan M, Wattigney WA, Srinivasan SR, Berenson GS. Serum bilirubin distribution and its relation to cardiovascular risk in children and young adults. Atherosclerosis 1997;131:107–13.
[26] Nakagami T, Toyomura K, Kinoshita T, Morisawa S. A beneficial role of bile pigments as an endogenous tissue protector: anticomplement effects of biliverdin and conjugated bilirubin. Biochim Biophys Acta 1993;1158:189–93.
[27] Tögl-Leimuller A, Egger G, Porta S. Albumin as a one way transport vehicle into sites of inflammation. Exp Pathol 1986;30:91–6.
[28] Galley HF, Thorton J, Howdle PD, Walker BE, Webster NR. Combination oral antioxidant supplementation reduces blood pressure. Clin Sci 1992;4:361–5.
[29] Bosma PJ, Chowdhury JR, Bakker C, et al. The genetic basis of the reduced expression of bilirubin UDP-glucuronosyltransferase 1 in Gilbert syndrome. N Engl J Med 1995;333:1171–5.
[30] (a) Douglas GA. Practical statistics for medical research. London: Chapman & Hall, 1994:93–6;
(b) Douglas GA. Practical statistics for medical research. London: Chapman & Hall, 1994:143–5;
(c) Douglas GA. Practical statistics for medical research. London: Chapman & Hall, 1994:325–36;
(d) Douglas GA. Practical statistics for medical research. London: Chapman & Hall, 1994:358–60.
[31] Anderson KM, Wilson PW, Odell PM, Kannel WB. An updated coronary risk profile. A statement for health professionals. Circulation 1991;83:356–62.
[32] Geizerová H, Widimský J. Některé aspekty primární prevence ICHS. Prakt Lék 1974;54:87–91.
[33] Ostrowska A, Tyszko P. Incidence of ischemic heart disease symptoms among residents of Plock and Kutno. Pol Tyg Lek 1994;49:566–9.
[34] Lipovetskii BM, Plavinskaia SI, Ilina GN. Clinicoepidemiological characteristics of ischemic heart disease in men of different age groups from the data of a single screening of a random population. Kardiologia 1983;23:81–5.
[35] Marcowitz PA, Shayna V, Horn RA, Hepner A, Armstrong WF. Value of dobutamine stress echocardiography in determining the prognosis of patients with known or suspect coronary artery disease. Am J Cardiol 1996;78:404–8.
[36] Eichholzer M, Stähelin HB, Gey F. Inverse correlation between essential antioxidants in plasma and subsequent risk to develop cancer, ischemic heart disease and stroke respectively: 12-year follow-up of the prospective basel study. Free Rad Aging 1992;62:398–410.
[37] Palgi A. Association between dietary changes and mortality rates; Israel 1949–1977; a trend free regression model. Am J Clin Nutr 1981;34:1569–83.
[38] Ginter E. Decline of coronary mortality in United States and vitamin C. Am J Clin Nutr 1979;32:511–2.
[39] Gey KF, Brubacher GB, Stähelin HB. Plasma levels of antioxidant vitamins in relation to ischemic heart disease and cancer. Am J Clin Nutr 1987;45:1368–77.
[40] Bowry VW, Stanley KK, Stocker R. High density lipoprotein is the major carrier of lipid hydroperoxides in human blood plasma from fasting donors. Proc Natl Acad Sci USA 1992;89:10316–20.
[41] Fluiter K, Sattler W, De Beer MC, Connell PM, van der Westhuyzen DR, van Berkel TJ. Scavenger receptor BI mediates the selective uptake of oxidized cholesterol esters by rat liver. J Biol Chem 1999;274:8893–9.
[42] Rice Evans CA, Miller NJ. Total antioxidant status in plasma and body fluids. Meth Enzymol 1994;234:279–93.
[43] Belanger S, Lavoie JC, Chessex P. Influence of bilirubin on the antioxidant capacity of plasma in newborn infants. Biol Neon 1997;71:233–8.
[44] Dailly E, Urien S, Barre J, Reinert P, Tillement JP. Role of bilirubin in the regulation of the total peroxyl radical trapping antioxidant activity of plasma in sickle cell disease. Biochem Biophys Res Commun 1998;248:303–6.
[45] Clark JE, Foresti R, Sarathchandra P, Kaur H, Green CJ, Motterlini R. Heme oxygenase-1-derived bilirubin ameliorates postischemic myocardial dysfunction. Am J Physiol Heart Circ Physiol 2000;278:H643–51.
[46] Raju VS, Imai N, Liang CS. Chamber-specific regulation of heme oxygenase-l (heat shock protein 32) in right-sided congestive heart failure. J Mol Cell Cardiol 1999;31:1581–9.
[47] Sharma HS, Manlik N, Gho BC, Das DK, Verdouw PD. Coordinated expression of heme oxygenase-1 and ubiquitin in the porcine heart subjected to ischemia and reperfusion. Mol Cell Biochem 1996;157:111–6.
[48] Raju VS, Maines MD. Renal ischemia/reperfusion up-regulates heme oxygenase-l (H5P32) expression and increases cGMP in rat heart. J Pharm Exp Ther 1996;277:1814–22.
[49] Ishizaka N, de Leon H, Laursen JB, et al. Angiotensin II-induced hypertension increases heme oxygenase-1 expression in rat aorta. Circulation 1997;96:1923–9.

[50] Hanckock WW, Buelow R, Sayegh MH, Turka LA. Antibody-induced transplant arteriosclerosis is prevented by graft expression of antioxidant and antiapoptotic genes. Nat Med 1998;4:1392–6.
[51] Sharma SC. Serum unconjugated bilirubin and free fatty acids in acute myocardial infarction and angina. J Assoc Physicians India 1985;33:473–5.
[52] Schwertner HA, Fischer JR Jr. Comparison of various lipid, lipoprotein, and bilirubin combinations as risk factors for predicting coronary artery disease. Atherosclerosis 2000;150:381–7.