The Relationship of the Anti-Oxidant Bilirubin with Free Thyroxine Is Modified by Insulin Resistance in Euthyroid Subjects

Petronella E. Deetman*, Stephan J. L. Bakker, Arjan J. Kwakernaak, Gerjan Navis, Robin P. F. Dullaart, on behalf of the PREVEND Study Group*

Department of Internal Medicine, University of Groningen, University Medical Center Groningen, The Netherlands

Abstract

**Background:** The strong anti-oxidative properties of bilirubin largely explain its cardioprotective effects. Insulin resistance is featured by low circulating bilirubin. Thyroid hormone affects both bilirubin generation and its biliary transport, but it is unknown whether circulating bilirubin is associated with thyroid function in euthyroid subjects. Aim is to determine relationships of bilirubin with TSH, free T4 and free T3 in euthyroid subjects without type 2 diabetes mellitus (T2DM), and to assess whether such a relationship would be modified by the degree of insulin resistance.

**Methods:** Total bilirubin, TSH, free T4, free T3, glucose, insulin, lipids and transaminases were measured in 1854 fasting euthyroid subjects without T2DM, recruited from the general population (PREVEND cohort). Insulin resistance was assessed by homeostasis model assessment.

**Results:** Bilirubin was positively related to free T4 ($b = 0.116, P<0.001$) and free T3 ($b = 0.078, P = 0.001$), but bilirubin was unrelated to TSH. The relationship of bilirubin with free T4 was modified by insulin resistance with a larger effect in more insulin resistant individuals (adjusted for age and sex; $b = 0.043, P = 0.056$ for interaction; additionally adjusted for smoking, alcohol intake, transaminases and total cholesterol ($b = 0.044, P = 0.044$ for interaction). The association of bilirubin with free T4 was also modified by high density lipoprotein cholesterol (age- and sex-adjusted: $b = 0.040, P = 0.072$).

**Conclusions:** Low bilirubin relates to low free T4 in euthyroid non-diabetic subjects. Low normal free T4 may particularly confer low bilirubin in more insulin resistant individuals.

Introduction

It is increasingly appreciated that endogenous bilirubin has strong anti-oxidative properties, which are attributed to its ability to scavenge peroxy radicals and to inhibit low density lipoprotein (LDL) oxidation [1]. Hence, the concept is emerging that bilirubin is involved in the pathogenesis of cardiometabolic disorders in which oxidative-stress is considered to play an important role [1–3]. In this line, low circulating levels of bilirubin levels have been documented to be associated with increased severity of atherosclerosis [4] and higher risk of lower limb amputation [5]. Low levels of circulating bilirubin have also been associated with increased cardiovascular and all-cause mortality in men [6]. In addition, intima media thickness, an established marker of subclinical atherosclerosis, is smaller in subjects with isolated hyperbilirubinemia [7]. Conversely, increased intima media thickness relates to low bilirubin in middle-aged subjects [8].

The importance of bilirubin for the development of atherosclerotic cardiovascular diseases underscores the relevance to delineate the metabolic factors that affect its metabolism in more detail. Thyroid hormones stimulate heme oxygenase-1 activity (HO-1), which is the main enzyme responsible for bilirubin production [9,10]. Furthermore, thyroid hormones downregulate the enzymatic activity of uridine 5’-diphospho-glucuronosyltransferase (UDP-GT), which stimulates bilirubin conjugation, thereby facilitating bilirubin excretion [11,12]. In agreement with the hypothesis that thyroid function represents a clinically relevant determinant of serum bilirubin metabolism, we have recently shown that low free $T_4$ levels confer decreased bilirubin levels in euthyroid patients with type 2 diabetes mellitus (T2DM) [13]. Of further interest, insulin resistance and the metabolic syndrome...
Table 1. Clinical characteristics, glucose, insulin, insulin resistance, lipids, transaminases, and thyroid hormones in 1854 subjects.

| Sex-stratified tertiles of bilirubin | 1 | 2 | 3 |
|--------------------------------------|---|---|---|
| **Men (n = 335)**                   |   |   |   |
| <7 μmol/L                            |   |   |   |
| Women (n = 354)                      |   |   |   |
| >7 μmol/L                            |   |   |   |
| **Women (n = 274)**                  |   |   |   |
| 6–7 μmol/L                           |   |   |   |
| 7–9 μmol/L                           |   |   |   |
| >9 μmol/L                            |   |   |   |

| Sex-stratified tertiles of bilirubin | 1 | 2 | 3 |
|--------------------------------------|---|---|---|
| Age (years)                          | 48±12 | 48±13 | 46±13 |
| BMI (kg/m²)                          | 26.5±4.5 | 25.7±4.4 | 25.1±3.9 |
| Alcohol                              | 0.077 | 0.001 |
| <10 gram per day (%)                 | 76 | 69 | 72 |
| ≥10 gram per day (%)                 | 24 | 31 | 28 |
| Current smoker (%)                   | 44 | 36 | 27 |
| Waist circumference in men (cm)      | 95±12 | 92±11 | 92±12 |
| Waist circumference in women (cm)    | 83±13 | 82±13 | 80±11 |
| Systolic blood pressure (mmHg)       | 129±21 | 129±19 | 126±20 |
| Diastolic blood pressure (mmHg)      | 74±9 | 74±9 | 73±10 |
| Glucose (mmol/L)                     | 4.4 (4.0–4.9) | 4.4 (4.0–4.8) | 4.2 (3.9–4.6) |
| Insulin (mU/L)                       | 9.0 (6.1–13.2) | 7.6 (5.5–11.1) | 7.1 (5.1–10.0) |
| HOMA-IR                              | 1.74 (1.15–2.76) | 1.47 (1.03–2.26) | 1.33 (0.92–1.94) |
| Total cholesterol (mmol/L)           | 5.8±1.16 | 5.60±1.16 | 5.4±1.08 |
| HDL cholesterol (mmol/L)             | 1.32±0.39 | 1.35±0.39 | 1.43±0.43 |
| Triglycerides (mmol/L)               | 1.24 (0.89–1.87) | 1.12 (0.84–1.56) | 1.01 (0.74–1.43) |
| Metabolic syndrome (%)               | 25 | 17 | 13 |
| TSH (mU/L)                           | 1.34 (0.98–1.87) | 1.28 (0.94–1.82) | 1.37 (1.00–1.85) |
| Free T₃ (pmol/L)                     | 12.66±1.69 | 13.08±1.79 | 13.04±1.77 |
| Free T₄ (pmol/L)                     | 3.69±0.62 | 3.72±0.61 | 3.80±0.62 |
| AST (U/L)                            | 24 (21–28) | 24 (21–29) | 25 (21–29) |
| ALT (U/L)                            | 20 (15–28) | 20 (16–28) | 20 (15–29) |

Data in mean ± SD or in median (interquartile range). BMI, body mass index; HOMA-IR, homeostasis model assessment-insulin resistance; HDL, high density lipoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; β, standardized regression coefficient. P-values for linear trend are shown. Bilirubin, glucose, insulin, HOMA-IR, triglycerides, TSH, AST and ALT were log transformed. doi:10.1371/journal.pone.0090886.t001

MetS are not only featured by low bilirubin levels, but also by low free T₄ [14,15]. In extension thereof, it may be hypothesized that a possible relationship of bilirubin with thyroid function among euthyroid subjects is influenced by insulin resistance. Against this background, the present study was initiated to test whether low plasma bilirubin is related to a lower thyroid functional status, body mass index (BMI), systolic and diastolic blood pressure, and waist circumference in men and women. The protocol of this study has been described elsewhere [16,17]. The medical ethics committee of the University Medical Center Groningen approved the study, and all participants gave written informed consent. A health questionnaire indicated that the participants had no history of liver disease.

For the current analysis, we excluded subjects not being euthyroid, subjects using thyroid hormones, anti-thyroid drugs and amiodarone, subjects with diabetes mellitus (as indicated by self-reported questionnaire, a physician diagnosis of diabetes, the use of oral glucose-lowering medication and/or elevated plasma glucose), as well as subjects in whom blood was not taken in the fasting state. Euthyroidism was defined as TSH, free T₄ and free T₃ levels within the reference range as provided by the manufacturer (see Laboratory analyses). We additionally excluded subjects with positive anti-thyroid peroxidase auto-antibodies (cut-off value: see Laboratory analyses). Applying these selection criteria, 1854 subjects were eligible for the current analyses.

Patient characteristics, including age, sex, alcohol use, smoking status, body mass index (BMI), systolic and diastolic blood pressure, and waist circumference were obtained. Blood was drawn after an overnight fasting period for measurement of free T₄, free T₃, TSH, bilirubin, glucose, insulin, total cholesterol, high density lipoprotein (HDL) cholesterol, triglycerides, aspartate aminotransferase (AST), and alanine aminotransferase (ALT).
Body mass index was defined as weight (kg) by height (m) squared. Alcohol consumption was recorded with one drink being assumed to contain 10 grams of alcohol. Insulin resistance was estimated using the Homeostasis Model Assessment-Insulin Resistance (HOMA-IR): glucose (mmol/L) × insulin (mU/L)/22.5 [18]. Three or more of the following criteria were required for categorization of subjects with MetS: waist circumference > 102 cm for men and > 80 cm for women, hypertension (blood pressure ≥130/85 mmHg or use of anti-hypertensive drugs), fasting triglycerides ≥1.70 mmol/L, fasting glucose ≥5.6 mmol/L, and HDL cholesterol < 1.03 mmol/L for men and < 1.29 mmol/L for women [19].

Laboratory analyses

Heparinized plasma and serum samples were stored at −80°C until analyses. Serum TSH (Architect; Abbott Laboratories, Abbott Park, IL, USA; reference range 0.35–4.94 mU/L), free T4 (AxSYM; Abbott Laboratories, Abbott Park, IL, USA; reference range 0.35–4.94 mU/L), free T3 (AxSYM; Abbott Laboratories, Abbott Park, IL, USA; reference range 1.23–5.55 pmol/L) were measured by microparticle enzyme immunoassay. Anti-thyroid peroxidase autoantibodies were demonstrated using commercially available automated enzyme linked immunoassays (Abbott Laboratories, Abbott Park, IL, USA; kit number 5F57). Anti-thyroid peroxidase autoantibodies were determined using a colorimetric assay (2,4-dicholoraniline reaction; Merck MEGA, Darmstadt, Germany). In healthy subjects, bilirubin is most abundantly present in serum in its unconjugated form [20].

The pyridoxal phosphate activation (Merck MEGA, Darmstadt, Germany). Serum total cholesterol and plasma glucose were measured using Kodak Ektachem dry chemistry (Eastman Kodak, Rochester, NY, USA). Serum triglycerides were measured enzymatically. HDL cholesterol was measured with a homogeneous method (direct HDL, AEROSET system; Abbott Laboratories, Abbott Park, IL, USA; no. 7D67). Insulin was measured by microparticle enzyme immunoassay (AxSYM; Abbott Laboratories, Abbott Park, IL, USA).

Table 2. Age- and sex-adjusted linear regression analyses demonstrating relationships of bilirubin with thyroid hormones, components of the metabolic syndrome, insulin, insulin resistance and total cholesterol.

| Total bilirubin (μmol/L) | β     | P-value |
|-------------------------|-------|---------|
| TSH (mU/L)              | −0.015  | 0.510   |
| Free T4 (pmol/L)        | 0.086  | <0.001  |
| Free T3 (pmol/L)        | 0.033  | 0.150   |
| Systolic blood pressure (mmHg) | −0.028  | 0.296   |
| Diastolic blood pressure (mmHg) | −0.058  | 0.026   |
| Waist circumference (cm) | −0.116  | <0.001  |
| Glucose (mmol/L)        | −0.055  | 0.022   |
| Insulin (mU/L)          | −0.144  | <0.001  |
| HOMA-IR                 | −0.143  | <0.001  |
| Total cholesterol (μmol/L) | −0.121  | <0.001  |
| HDL cholesterol (μmol/L) | 0.154   | <0.001  |
| Triglycerides (mmol/L)  | −0.176  | <0.001  |

HDL, high density lipoprotein; HOMA-IR, homeostasis model assessment-insulin resistance; β, standardized regression coefficient. Bilirubin, TSH, glucose, insulin and HOMA-IR and triglycerides were log transformed.

doi:10.1371/journal.pone.0090886.t002

Results

A total of 1854 subjects (age 47±13, 50% men) participated in this study. Median bilirubin concentration was 8 (6–10) μmol/L in men and 6 (5–8) μmol/L in women (P<0.001). Clinical and laboratory characteristics of the study population are, therefore, shown according to sex-stratified tertiles of bilirubin (Table 1). Angiotensin converting enzyme inhibitors (ACEi) or angiotensin receptor blockers (ARB’s) were used by 56 subjects (3%); 46 subjects (3%) used lipid lowering drugs (mainly statins).

In univariable analyses, bilirubin was inversely related to age, BMI, smoking status, waist circumference, insulin, HOMA-IR, total cholesterol, triglycerides, and the presence of metabolic syndrome (Table 1). We also found positive relationships of bilirubin with free T4 and free T3, alcohol use, ALT, and AST. Bilirubin was not associated with blood pressure, glucose, HDL cholesterol and TSH. There were no interactions of sex with free T4, free T3 and TSH on bilirubin (P>0.29 for all; data not shown). In age- and sex-adjusted linear regression analyses (Table 2), bilirubin was positively associated with free T4, but there were no significant associations of bilirubin with free T3 and TSH. Bilirubin was inversely associated with diastolic blood pressure, waist circumference, glucose, insulin, HOMA-IR, total cholesterol, HDL cholesterol and triglycerides in age- and sex-adjusted analyses (Table 2).

We then tested whether the relationship of bilirubin with free T4 was modified by HOMA-IR, fasting insulin, individual MetS

Statistical analyses

Data analyses were performed using SPSS (version 20.0, SPSS Inc. Chicago, IL, USA). Normally distributed data are given as mean ± standard deviation (SD) and non-parametrically distributed data are presented as median (interquartile range, IQR). Categorical variables are given as percentages. Differences in bilirubin concentration between sexes were determined by Mann-Whitney U-test. Characteristics of the study population are presented according to sex-stratified tertiles of bilirubin. Univariable linear regression analysis was used to test for linear trends across tertiles of bilirubin. Multivariable linear regression analyses were used to determine the extent to which bilirubin is related to thyroid function, components of the metabolic syndrome, insulin, HOMA-IR and transaminases. To this end, logarithmically transformed values of bilirubin, glucose, insulin, HOMA-IR, triglycerides, TSH and transaminases were used. Multivariable models were all age- and sex-adjusted. Before calculating interaction terms, the continuous variable of interest were centered to the mean by subtracting the group mean value from individual values. This was done in order to avoid multicollinearity [24,25]. Interaction terms were considered statistically significant at P-values <0.10, as proposed by Selvin [26] and recommended by the Food and Drug Administration authorities [27]. Otherwise, two-sided P-values <0.05 were considered significant.
components, and total cholesterol. The relationship of bilirubin with free T4 was significantly modified by HOMA-IR ($\beta = 0.043$, $P = 0.056$ for interaction; Table 3) and by plasma insulin ($\beta = 0.040$, $P = 0.072$ for interaction; Table S1). The effect-modification of free T4 by HOMA-IR was independent of potential confounding factors including smoking, alcohol use $\leq 10$ gram/day, AST, ALT and total cholesterol ($\beta = 0.044$, $P = 0.044$ for interaction; Table 3). Figure 1 provides a graphical presentation of the modification of the effect of free T4 on bilirubin by HOMA-IR. As shown in Table S1, there were no significant modifications of the effect of free T4 on bilirubin by systolic blood pressure, diastolic blood pressure, waist circumference, glucose, total cholesterol, or triglycerides in age- and sex-adjusted analyses ($P > 0.27$ for all), but there was a significant modification of the effect of free T4 on bilirubin by HDL cholesterol ($\beta = 0.040$, $P = 0.072$). In addition, there were no significant modifications of a potential effect of free T3 on bilirubin by HOMA-IR, fasting insulin, components of the metabolic syndrome, and total cholesterol ($P > 0.32$ for all; data not shown).

Secondary analyses were performed after exclusion of subjects using lipid lowering drugs, ACEi and ARB’s. In the remaining subjects ($n = 1759$), there was again an age- and sex-adjusted positive relationship of bilirubin with free T4 ($\beta = 0.094$, $P < 0.001$). Furthermore, the interaction of free T4 with HOMA-IR on bilirubin was also significant in these analyses ($\beta = 0.050$, $P = 0.030$ for interaction), and remained significant after further adjustment for alcohol intake, transaminases and total cholesterol ($\beta = 0.056$, $P = 0.013$ for interaction).

**Discussion**

To our knowledge, this is the first report on an independent positive relationship of total bilirubin with free T4 in a large group of euthyroid, non-diabetic individuals recruited from the general population. Of note, multivariable linear regression analyses demonstrated a significant positive modification of the effect of free T4 on bilirubin by insulin resistance as quantified by HOMA-IR. This effect-modification remained essentially unaltered after controlling for potential confounders, including smoking, alcohol, transaminases, and total cholesterol. Our results, therefore, are in concert with the hypothesis that low-normal thyroid function may confer lower circulating bilirubin levels, especially in insulin resistant individuals. In addition, the effect of free T4 on bilirubin was modified by the HDL cholesterol concentration.

We recently documented a positive relationship of circulating levels of bilirubin with free T4 in euthyroid T2DM subjects [13]. In that report, bilirubin was not significantly correlated with free T3 ($\beta = 0.027$, $P = 0.52$).
relevance that the effect of free T4 on bilirubin was modified by oxidative capacity by thyroid function [29], it is also of potential thyroid function. In view of the strong anti-oxidative properties of could represent a mechanism linking low bilirubin to low normal se

effect modification was not observed with plasma glucose, raising

was most pronounced in the most insulin resistant subjects. This

modification of the effect of free T4 on bilirubin by HOMA-IR

be more strongly associated with HOMA-IR and insulin than with

non-diabetic subjects. In the current study, we found bilirubin to

enhanced UDP-glucuronyltransferase activity and bilirubin excre-

tion [36]. Further study is required to more precisely delineate the

mechanisms responsible for the alleged effects of thyroid functional

status on bilirubin metabolism. Moreover, it remains to be

established why bilirubin was related to free T4 (and in univariable analysis also to free T3) but not to the TSH level, extending our previous report showing relationships of plasma lipids with free thyroid hormone levels rather than with TSH [13].

Several other methodological issues and limitations of the present study warrant consideration. First, euthyroidism was strictly defined as levels of free T4, free T3 and TSH within the assay-specific reference range as provided by the manufacturer. We also excluded subjects with positive anti-thyroid peroxidase auto-antibodies. This was done to reduce possible bias in the relationship of free thyroid hormone levels with TSH in subjects with very early stages of autoimmune thyroid dysfunction as much as possible. Second, we performed a cross-sectional study. Thus, cause-effect relationships cannot be established with certainty. However, bilirubin has been shown not to influence the set-point of the pituitary-thyroid axis [37], strongly suggesting that low bilirubin levels by themselves are unlikely to lower thyroid function. Third, statin treatment has been reported to decrease bilirubin levels [38], and to increase plasma glucose [39], whereas ACEi or ARB’s are likely to inhibit oxidative stress [40] and to improve insulin sensitivity [41]. In primary analyses, we did not exclude subjects using lipid lowering drugs or individuals using ACEi or ARB’s. Instead, we carried out a secondary analysis after exclusion of subjects using these medications. This secondary analysis showed an essentially unaltered relationship of bilirubin with free T4 and a similar interaction of free T4 with HOMA-IR on bilirubin.

In conclusion, the current study shows an independent relationship of low bilirubin with low free T4 in euthyroid subjects. Low normal free T4 may particularly confer low bilirubin in more insulin resistant individuals. Since bilirubin is a potent endogenous anti-oxidant, it is plausible to speculate that low normal thyroid functional status could enhance atherosclerosis susceptibility in the context of insulin resistance.

**Supporting Information**

Table S1 Multivariable linear regression models demonstrating interactions of free T4 with metabolic syndrome components on bilirubin.
Acknowledgments

In addition to the authors, the PREVEND investigators are, from the university medical center Groningen, the Netherlands: de Jong PE, Gansevoort RT, Navis GJ, Bakker SJL (Dept. of Nephrology); van Veldhuijzen DJ, van Vooys AA, van der Harst P (Dept. of Cardiology); van Gilst WH, de Boer R (Dept. of Experimental Cardiology); Stolk RP, Hillegaart KH (Dept. of Clinical Epidemiology); Stolk RP, Dullaart RPF, Wollensak BHR (Dept. of Endocrinology); Slaets JPJ, Izaak G (Dept. of Geriatric Medicine) Gans ROB (Dept. of Internal Medicine); van de Berg PB, de Jong-van den Berg LT, Postma MJ, Visser ST (Dept. of Pharmacoepidemiology and Pharmacoeconomics). Lead author: Bakker SJL, s.j.l bakker @umcg.nl.

Author Contributions

Conceived and designed the experiments: PED SJLB GJN RPFD. Wrote the paper: PED AKJ RPFD. Initiation of the study: SJLB RPFD. Intellectual contributions: GJN. Supervision of data analyses, study planning: RPFD.

References

1. Vitek L, Schwertner HA (2007) The heme catabolic pathway and its protective effects on oxidative stress-mediated diseases. Adv Clin Chem 43: 1–57.
2. Vitek L (2012) The role of bilirubin in diabetes, metabolic syndrome, and cardiovascular diseases. Front Pharmacol 3: 55.
3. Oda E, Aizawa Y (2013) Total bilirubin is inversely associated with metabolic syndrome but not a risk factor for metabolic syndrome in Japanese and men. Acta Diabetol 50: 417–422.
4. Novotny L, Vitek L (2003) Inverse relationship between serum bilirubin and athero-occlusiveness in men: A meta-analysis of published studies. Exp Biol Med (Maywood) 228: 560–566.
5. Chan KH, O'Connell RL, Sullivan DR, Hoffmann LS, Rajamani K, et al. (2013) Plasma total bilirubin levels predict amputation events in type 2 diabetes mellitus: The fenofibrate intervention and event lowering in diabetes (FIELD) study. Diabetologia 56: 724–736.
6. Aijia R, Lee DC, Sui X, Church TS, Steven NB (2011) Usefulness of serum bilirubin and cardiorespiratory fitness as predictors of mortality in men. Am J Cardiol 108: 1430–1442.
7. Vitek L, Novotny L, Sperli M, Holaj R, Spacil J (2006) The inverse association of elevated serum bilirubin levels with subclinical carotid atherosclerosis. Cerebrovasc Dis 21: 401–414.
8. Dullaart RPF, Kappelle PJ, de Vries R (2012) Lower carotid intima media thickness is predicted by higher serum bilirubin in both non-diabetic and type 2 diabetic subjects. Clin Chim Acta 414: 161–165.
9. Smith TJ, Drummond GS (1991) Retinoic acid can enhance the stimulation by thyroid hormone of heme oxygenase activity in the liver of thyroidectomized rats. Biochim Biophys Acta 1073: 119–122.
10. Li F, Lu S, Zhu K, Zhou Z, Ma L, et al. (2011) Heme oxygenase-1 is induced by thyroid hormone and involved in thyroid hormone preconditioning-induced protection against renal warm ischemia in rat. Mol Cell Endocrinol 339: 54–62.
11. Gartner LM, Arias IM (1972) Hormonal control of hepatic bilirubin transport and conjugation. Am J Physiol 222: 1091–1099.
12. Van Steenbergen W, Fevery J, De Vos R, Leyten R, Heirwegh KP, et al. (1989) Thyroid hormones and the hepatic handling of bilirubin. I. effects of hypothyroidism and hyperthyroidism on the hepatic transport of bilirubin mono- and diglucuronides in the wistar rat. Hepatology 9: 314–321.
13. Deetman PE, Kwakernaak AJ, Bakker SJ, Dullaart RP (2013) Low normal free thyroxine levels decrease serum bilirubin in type 2 diabetes mellitus. Thyroid. In press.
14. Roos A, Bakker SJ, Links TP, Gans RO, Wollensak BHR (2007) Thyroid function is associated with components of the metabolic syndrome in euthyroid subjects. J Clin Endocrinol Metab 92: 491–496.
15. Heima NE, Eerkhof EM, Oosterwijk MM, Lips PT, van Schoor NM, et al. (2012) Thyroid function and the metabolic syndrome in older persons: A population-based study. Eur J Endocrinol 168: 59–65.
16. Hillegaart HL, Janssen WM, Bak AA, Diercks GF, Grobbee DE, et al. (2001) Microalbuminuria is common, also in a nondiabetic, nonhyperensive population, and an independent indicator of cardiovascular risk factors and cardiovascular morbidity. J Intern Med 249: 519–526.
17. Pinto-Sierou SJ, Janssen WM, Hillegaart HL, Navis G, De Zeeuw D, et al. (2000) Urinary albumin excretion is associated with renal functional abnormalities in a non-diabetic population. J Am Soc Nephrol 11: 1882–1888.
18. Mathews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, et al. (1985) Homeostasis model assessment: Insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 28: 412–419.
19. Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (2001) Executive summary of the third report of the national cholesterol education program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (adult treatment panel III). JAMA 285: 2486–2497.
20. Tiodale WA, Klaasen C, Kinsey SD (1989) The significance of the direct-reacting fraction of serum bilirubin in hemolytic jaundice. Am J Med 26: 214–227.
21. Zelle DM, Deetman N, Alkhala A, Navis G, Bakker SJ (2011) Support for a protective effect of bilirubin on diabetic nephropathy in humans. Kidney Int 79: 698; author reply 698–7.
22. Kasuki N, Karagiansis A, Mikhaldissi DP (2013) Diabetes, bilirubin and amputations: Is there a link? Diabetologia 56: 683–685.
23. Deetman PE, Zelle DM, Homan van de Heule JJ, Navis GJ, Gans RO, et al. (2012) Plasma bilirubin and late graft failure in renal transplant recipients. Transpl Int 25: 876–881.
24. Shahi G (2011) Clarifying the role of mean centering in multicollinearity of interaction effects. Br J Math Stat Psychol 64: 462–477.
25. Keavney BC, Blassey CM (2004) Centering in regression analyses: A strategy to prevent errors in statistical inference. Int J Methods Psychiatr Res 13: 141–151.
26. Selvin S (1996) Statistical analysis of epidemiological data. New York: Oxford University Press.
27. Lu M, Lyden PD, Brott TG, Hamilton S, Broderick JP, et al. (2005) Beyond subgroup analysis: Improving the clinical interpretation of treatment effects in stroke research. J Neurosci Methods 143: 209–216.
28. Triolo M, Amema W, Dullaart RP, Tijge UJ (2013) Assessing the functional properties of high-density lipoproteins: An emerging concept in cardiovascular research. Biomark Med 7: 475–477.
29. Triolo M, de Boer JT, Amema W, Kwakernaak AJ, Tijge UJ, et al. (2013) Low normal free T4 confers decreased high-density lipoprotein antioxidant functionality in the context of hyperglycaemia. Clin Endocrinol (Oxf) 79: 416–423.
30. Ferder L, Inserra F, Martinez-Maldonado M (2006) Inflammation and the metabolic syndrome: Role of adiponectin B and oxidative stress. Curr Hypertens Rep 8: 191–198.
31. Duntas LH (2002) Thyroid disease and lipid. Thyroid 12: 287–293.
32. Chen SJ, Yen CH, Huang YC, Lee BJ, Huai S, et al. (2012) Relationships between inflammation, adiponectin, and oxidative stress in metabolic syndrome. PLoS One 7: e45693.
33. Harrison EM, McNally SJ, Devey L, Garden OJ, Ross JA, et al. (2006) Insulin induces heme oxygenase-1 through the phosphatidylinositol 3-kinase/Akt pathway and the Nrf2 transcription factor in renal cells. PLoS B 273: 2345–2356.
34. Angeli K, Thräflatus D, Ben I, Gaitaniak C (2011) Insulin-induced oxidative stress up-regulates heme oxygenase-1 via diverse signaling cascades in the C2 skeletal myoblast cell line. Endocrinology 152: 1274–1283.
35. Bao W, Song F, Li X, Ren S, Yang W, et al. (2010) Plasma heme oxygenase-1 concentration is elevated in individuals with type 2 diabetes mellitus. PLoS One 5: e12371.
36. Tunon MJ, Gonzalez F, Garcia-Pardo LA, Gonzalez J (1991) Hepatic transport of bilirubin in rats with streptozotocin-induced diabetes. J Hepatol 15: 71–77.
37. Wassen FW, Moerings EP, van Toor H, Hemmema G, Évers ME (2000) Thyroid hormone uptake in cultured rat anterior pituitary cells: Effects of energy status and bilirubin. J Endocrinol 165: 599–606.
38. Ong KL, Wu BJ, Cheung BM, Bartter PJ, Rye KA (2011) Association of lower total bilirubin level with statin usage: The united states national health and nutrition examination survey 1999–2008. Atherosclerosis 219: 728–733.
39. Preiss D, Seshasai SR, Welsh P, Murphy SA, Ho JE, et al. (2011) Risk of incident type 2 diabetes mellitus: The fenofibrate intervention and event lowering in diabetes (FIELD) study. Lancet 378: 2065–2072.
40. Muscogiuri G, Chavez AO, Gastaldelli A, Perez L, Tripathy D, et al. (2008) The crosstalk between insulin and renin-angiotensin-aldosterone signaling systems and its effect on glucose metabolism and diabetes prevention. Curr Vasc Pharmacol 6: 301–312.