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Mild hyperbilirubinaemia as an endogenous mitigator of overweight and obesity: Implications for improved metabolic health

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ABSTRACT

Background and aims: Mild endogenous elevation of unconjugated bilirubin (UCB) as seen in Gilbert's syndrome (GS), might mitigate cardiovascular disease (CVD) risk factors including overweight/obesity. This study aimed to determine whether hyperbilirubinaemia is linked to improved anthropometric data and lipid profile.
Methods: Our study considered GS and age-/gender-matched healthy controls (n = 248). Additionally, obese female type 2 diabetic patients (DM2) (n = 26) were included as a "disease control group".
Results: BMI, hip circumference (HC), and lipid profile were significantly lower in GS. UCB was inversely correlated with BMI ($p < 0.001$), HC as well as with fat mass (FM) and lipid variables ($p < 0.05$). Moreover, DM2 patients had significantly lower UCB compared to GS and healthy controls. Older GS subjects (≥35 years) had significantly reduced anthropometric data and improved lipid profile.
Conclusions: Our results propose that the health promoting potential of mild hyperbilirubinaemia may extend to protection from age-related weight gain and dyslipidaemia.

1. Introduction

Obesity is a life-threatening public health issue and is associated with chronic diseases including type 2 diabetes mellitus (DM2) and cardiovascular disease (CVD) [1]. Risk factors of CVD and premature mortality include unfavorable alterations in body weight, body composition and lipid metabolism [2,3]. These risk factors, however, seem to be less prevalent in those 5–10% of the general population presenting with Gilbert's syndrome (GS). This hereditary condition is based on various underlying promoter polymorphisms in the UDP glucuronosyltransferase 1A1 *(UGT1A1)* gene (e.g. *UGT1A1*28*) leading to a reduced conjugating activity of this enzyme, phenotypically resulting in a mild increase in unconjugated bilirubin (UCB) (total bilirubin >17.1 μmol/l) [4].

Scattered data have shown an inverse association of UCB with weight, body mass index (BMI), and abdominal obesity, suggesting it may play a role in CVD/DM2 risk mitigation, especially in the elderly [5–7]. In that respect, Andersson et al. demonstrated that weight loss (over 4 weeks) was associated with an increase in bilirubin, however, this period was too short to examine cardiovascular risk factors (overweight, hyperlipidaemia or DM2) [8]. Phenotypically, the Framingham heart study showed that higher serum bilirubin was associated with lower risk of CVD [9] and a different study (meta-analysis, ~15,000 men), demonstrated that each 1 μmol/l increase in serum bilirubin was associated with a 6.5% CVD risk reduction [10].

This study aimed to investigate whether (i) hyperbilirubinaemia is correlated to CVD risk factors (anthropometric and lipid data), (ii) these effects are age and gender dependent, and (iii) UCB is

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associated with metabolic changes in a high risk CVD group of DM2 subjects.

2. Materials and methods

2.1. Data collection

A total of 248 subjects were included in this study. ALT (alanine transferase), AST (aspartate aminotransferase), γ-GT (γ-glutamyl transferase), LDH (lactate dehydrogenase), ALP (alkaline phosphatase), haemoglobin and haematocrit were measured at initial screening examinations. Exclusion criteria for these studies included age <20 yrs, pregnancy, chronic disease, alcoholism (>7 standard drinks/week), smoking (>1 cigarettes/day), excessive physical activity (>10 h/week) and intake of any medication or supplements [11,12]. Allocation to the GS or healthy control group (HCG) was based on total serum bilirubin (cut-off: 17.1 μmol/l or = 1 mg/dl) [13]. Both groups (n = 124 each) were matched for gender and age. Furthermore, the study population was divided into older and younger subsets (cut-off: 35 yrs). In addition to this main study population, female DM2 patients (n = 26) were introduced as an age-matched "disease control group" to both older female groups (GS and HCG).

All anthropometric measurements were obtained from participants who were barefooted and lightly dressed in the mornings of the study days. Body height was measured by stadiometer (model 214, Seca) to the nearest 0.5 cm and body weight using standard analogous scales (Selecta 791, Seca). Waist circumference (WC) and hip circumference (HC) were measured by tape (model 203, Seca). BMI and WHR (waist-to-hip ratio) were calculated using the equations BMI = kg/m^2 and WHR = WC/HC, respectively. To determine body fat mass (FM), Bioelectric Impedance Analysis (BIA) was used (BIA Analyser 2000-S, Data-Input GmbH, Germany).

For each subject, an overnight fasting blood sample was collected into serum tubes. Samples were kept cool and protected from light until being analysed or aliquoted (samples aliquots were stored at −80 °C for further analyses). Serum total cholesterol (TC), triglycerides (TG), high density lipoprotein (HDL) and low density lipoprotein (LDL) were analysed using clinical routine diagnostic tests.

Serum UCB was analysed following a well-established high-performance liquid chromatography (HPLC) protocol [12,13]. Briefly, serum UCB was measured by HPLC (Merck, Hitachi, LaChrom), equipped with a Fortis C18 HPLC column (4.6 × 150 mm, 3 μm), a Phenomenex SecurityGuard™ cartridges for C18 HPLC columns (4 × 3 mm) and a photodiode array detector (PDA, Shimadzu). All studies had been approved by local ethical committees (274/2010, 1164/2014, 1987/2013) [11,12,14] and were conducted in accordance with the Declaration of Helsinki. All participants provided signed informed consent.

2.2. Statistical analysis

Statistical analyses were performed using SPSS (IBM statistics, Version 23.0). Prior to analysis, missing values had been excluded. A p <0 .05 was considered significant for all procedures.

The Kolmogorov Smirnov test was used to determine data distribution. For comparison of two groups, the independent samples t-test (parametric data) or Mann-Whitney U test (non-parametric data) were used. For comparison of three groups, one-way analysis of variance (ANOVA) with Bonferroni adjustment (parametric data), Kruskal-Wallis H-test with pairwise comparisons (non-parametric data) and Chi-square test were performed. Analysis of covariance (ANCOVA) was performed where needed. Correlation between variables were analysed by Pearson or Spearman correlation. Subsequently, stepwise multiple logistic regression analysis was performed separately for both the younger and the older subgroups, to identify significant predictors for selected dependent variables.

3. Results

3.1. Age-matched GS (Gilbert's syndrome) group the HCG (healthy control group)

The GS group had higher serum UCB (30.7 ± 11.4 *vs.* 8.7 ± 3.6, p < .001), lower BMI (8%, p < 0.001) and HC (4%, p <0 .01) compared to the HCG. 25% of GS and 46% of the HCG were overweight/obese (p <0 .01). Further biologically relevant yet non-significant differences in WC, abdominal circumference (AC), WHR and the FM were found between the groups (Table 1).

Lipid parameters were significantly lower in GS compared to the HCG (p <0 .05): 7% LDL; 8% TG; 6% TC; 12% TC/HDL; 15% LDL/HDL (Table 1). For the entire study population (n = 248), UCB was inversely correlated with BMI (r = −0.268, p <0.001), HC (r = −0.249, p <0.01) and FM (r = −0.226, p <0.05) and all lipid variables (p < 0.01) except HDL.

To back up those bivariate relationships, stepwise linear regression analysis was performed for both age subgroups (</≥ 35 yrs, Supplementary Table 3).

In the older group, we found UCB serum concentrations to explain variation in FM (adjusted R square = 0.207, p = 0.001) and TG levels (adjusted R square = 0.089, p = 0.003). No associations were found in the younger group. In this group, furthermore, no significant differences in anthropometric data (except BMI) and lipid profile were found between GS and the HCG. However, older GS subjects (≥35 yrs), had significantly improved anthropometric and lipid variables (13% BMI, 6% HC, and 26% FM, 15% LDL and TG, 9% TC, 20% TC/HDL, 28% LDL/HDL and 13% greater HDL) relative to their HCG counterparts (p < 0.05; Table 1). The prevalence of overweight/obesity among older GS (35%) was nearly half that of their controls (69%, p < 0.01).

3.2. Age-matched females (≥35yrs.) in the GS (Gilbert's syndrome), HCG (healthy control group) and DM2 (type 2 diabetic patients) groups

In addition to GS and the HCG, an older female age-matched disease group with DM2 was included (n = 26 each group) (Fig. 1). The mean age in GS, HCG and DM2 was 53.2 ± 9.8|52.5 ± 10.4|54.5 ± 8.6, and the mean fasting plasma glucose and HbA1C were 4.6 ± 0.5 mmol/l, 4.7 ± 0.8%|4.9 ± 0.9 mmol/1, 4.8 ± 0.9%|9.04 ± 2.3 mmol/l, 7.8 ± 0.8%, respectively.

UCB was significantly lower in DM2 compared to the HCG and GS (3.6 ± 2|7.2 ± 3|25.8 ± 7 μmol/l; p < 0.05), whereas BMI (36.5 ± 9|26.9 ± 4|23.0 ± 4 kg/m^2, p < 0.001), WC (105.0 ± 14 |86.9 ± 14|77.9 ± 11 cm; p < 0.001), HC (119.2 ± 16|101.9 ± 10| 96.1 ± 8 cm; p < 0.001), and AC (114.1 ± 17|90.1 ± 13|83.4 ± 9; p < 0.001) were significantly greater. Relative to GS subjects (but not to the HCG), the WHR of DM2 was significantly increased (0.88 ± 0.1|0.80 ± 0.1; p = 0.003). The prevalence of overweight/obesity in GS, the HCG and DM2 was 25%, 69% and 100%, respectively (p < 0.001).

To correct for the intake of statins in 15 DM2 patients, lipid parameters were analysed using ANCOVA with statin-intake as covariate. HDL was significantly lower in DM2 compared to GS (p < 0.001) and tended to be lower relative to the HCG (p = 0.075). The HCG had the highest LDL, which was significantly increased compared to GS (p = 0.013) but not to DM2 (due to the effect of statins). TG were elevated in DM2 compared to the HCG (p = 0.042)

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