Handgrip Strength Is Positively Associated with Mildly Elevated Serum Bilirubin Levels among Community-Dwelling Adults

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Handgrip strength (HGS) is a useful measure of health-related quality of life and general muscle strength. Serum total bilirubin (T-B) may present potential beneficial effects in preventing oxidative changes which are associated with a risk of metabolic syndrome and the development of cardiovascular disease. Limited information is available regarding whether HGS is an independent confounding factor for serum T-B. The study participants were 214 men aged 71 ± 8 (mean ± standard deviation) years and 302 women aged 71 ± 7 years that were enrolled consecutively from among paticipants aged ≥ 50 years through an annual check-up process. We evaluated the relationship between serum T-B and confounding factors within each sex. HGS related significantly with serum T-B in both men (r = 0.156, *p* = 0.023) and women (r = 0.173, *p* = 0.003). Multiple linear regression analysis showed that in men, HGS (β = 0.173) as well as smoking status (β = −0.147), exercise habit (β = 0.138), low-density lipoprotein cholesterol (β = 0.146), and hemoglobin A1c (HbA1c) (β = −0.198) were significantly and independently associated with serum T-B. In women, HGS (β = 0.159) as well as smoking status (β = −0.116), high-density lipoprotein cholesterol (β = 0.159), and HbA1c (β = −0.161) were significantly and independently associated with serum T-B. Multivariate-adjusted serum T-B levels were significantly lower in subjects with the lowest HGS level in both sexes. Increased HGS is strongly associated with increased serum T-B, independent of confounding factors in both sexes.

Introduction

Bilirubin comprises an open chain of four pyrrole-like rings (tetrapyrrole) and a breakdown product of normal heme catabolism. Approximately 80% of the total bilirubin (T-B) is derived from hemoglobin degradation and most of the remaining 20% is derived from other hemo proteins. Excessive production of bilirubin above the threshold concentration is toxic to the human system, and is excreted in the urinary system and gastrointestinal tract (Nag et al. 2009). While, in adults serum bilirubin may present potential beneficial effects in preventing oxidative changes (Stocker et al. 1987) as scavengers of lipid peroxyl radicals and human LDL against lipid peroxidation (Wu et al. 1994; Yamaguchi et al. 1996). Epidemiological studies have generally suggested that high bilirubin levels are associated with a reduced risk of metabolic syndrome (Wu et al. 2011) and cardiovascular disease (CVD) (Novotný and Vítek 2003; McArdle et al. 2012), and therefore against the development of vascular disease.

Handgrip strength (HGS) was very strongly correlated with total muscle strength with correlation coefficients ranging from 0.736 to 0.890, and the correlation remained strong with adjustments for body weight (BW) (0.485-0.564). This demonstrates that HGS can be used as a valid measurement to provide rapid indication of someone's general muscle strength (Wind et al. 2010). In healthy older adults, a strong relationship between health-related quality of life (QOL) and muscle function in relation to HGS has been reported (Sayer et al. 2006; Jakobsen et al. 2010). Prospective studies suggest that decreased HGS is a risk of increased mortality due to CVD and cancer in men, even when with adjustments for confounding factors of muscle mass and body mass index (BMI) (Rantanen et al. 2003; Gale et al. 2007). HGS may also be a more useful indicator of physical frailty for older adults of similar age than chronological age alone (Syddall et al. 2003).

Lifelong physical exercise delays age-related loss of skeletal muscle mass (Zampieri et al. 2015), and upregulates antioxidant systems and induce many health benefits such as a reduced threat of all-cause mortality along with a reduced risk of type 2 diabetes, CVD, and cancer

(Warburton et al. 2006). While, it has been demonstrated that intense exercise increases blood bilirubin levels which are strong antioxidants (Stocker et al. 1987; Fallon et al. 1999; McKenzie et al. 2007), the effect of chronic exercise on bilirubin levels in skeletal muscle remains controversial. To our knowledge, limited information is available regarding whether HGS is an independent confounding factor for serum T-B among men and women (Rantanen et al. 2003).

We investigated the relationship between serum T-B and confounding factors such as age, BMI, habits, lipids, and hyperglycemia within each sex, and whether there is an independent association between HGS and serum T-B, using cross-sectional data from community-dwelling adults aged ≥ 50 years.

Materials and Methods

Subjects

The study participants aged ≥ 50 years were selected through a community-based annual check-up process from the Nomura health and welfare center in a rural town where the aging rate is 40%, and located in Ehime prefecture, Japan. Exercise habits of the participants, information on present conditions, medical history, and medications (e.g., antihypertensive, antidyslipidemic, and hypoglycemic medication) were obtained using structured interviews and questionnaires. For all participants, blood samples and HGS test were taken after an overnight fast. Participants with serum T-B > 2.0 mg/dL or alanine transaminase (ALT) ≥ 100 IU/L or gamma glutamyl transpeptidase (GGT) ≥ 100 IU/L were excluded to avoid confounding factors because the probability of potential Gilbert syndrome and hepatobiliary disease is high. Thus, 516 participants (men, 214/women, 302) were enrolled in the study. The study complies with the Declaration of Helsinki, and was approved by the ethics committee of Ehime University School of Medicine with written informed consent obtained from each subject (IRB: 1402009). Informed consent was obtained from all participants.

Evaluation of Risk Factors

The data collected using clinical files included information on demographic characteristics and risk factors. Body mass index (BMI) was calculated as weight in kilograms divided by the square of the height in meters. The cumulative exposure to smoking status was defined as the number of packs of cigarette smoked per day multiplied by the number of years (pack-year), and the participants were classified as never smokers, past smokers, light smokers (< 20 pack-year) and heavy smokers (≥ 20 pack-year) based on pack-year of smoking. Alcohol habit was measured using the Japanese liquor unit in which a unit corresponds to 23 g of ethanol, and the participants were classified as never drinkers, occasional drinkers, daily light drinker (< 2 unit/day), and daily heavy drinkers (≥ 2 unit/day). Triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), fasting blood glucose (FBG), hemoglobin A1c (HbA1c), immunoreactive insulin (IRI), and serum T-B were measured in the fasted state. Insulin resistance was defined as the homeostatic model assessment for insulin resistance (HOMA-IR). HOMA-IR was calculated from FBG and IRI levels using the following formula: (FBG (mg/dL) X IRI (mU/mL))/405 (Matthews et al. 1985).

HGS test

This test was used to measure the isometric strength of the hand and forearm muscles using Takei Digital Hand Grip. Participants were required to hold the dynamometer in the hand to be tested, with the arm at right angles and the elbow by the side of the body. The handle of the dynamometer is adjusted if required - the base should rest on the first metacarpal (heel of palm), while the handle should rest on middle of four fingers. Participants were asked to squeeze the dynamometer with maximum isometric effort, which is maintained for about 5 s. During the contact, no other body movement is allowed (Handgrip strength test 2014). A total of two right and left trails were made and the mean of four measurements was used for the analysis.

Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics Version 21 software (Statistical Package for Social Science Japan, Inc., Tokyo, Japan). All values are expressed as the mean ± standard deviation (SD), and for parameters with non-normal distribution (such as TG, HbA1c, HOMA-IR, serum T-B, and HGS), the data are shown as median (interquartile range) values. In all the analyses, parameters with non-normal distributions were used after log-transformation. The differences among the groups were analyzed by Student's t-test for continuous data and χ^2 test for categorical data, respectively. Pearson's correlations were calculated in order to characterize the relationships between various confounding factors and serum T-B. Forward entry stepwise multiple linear regression analysis (*p*-value for entry was < 0.05 and for exit was > 0.10) was used to evaluate the contribution of each confounding factor to serum T-B. ANCOVA was performed using a general linear model approach to determine the association between confounding factors and serum T-B. In these analyses, serum T-B was the dependent variable, the four categories of HGS were the fixed factors, and all confounding factors in model 2 of Table 4 were added as covariates. A value of $p < 0.05$ was considered significant.

Results

Characteristics of subjects

Table 1 shows the characteristics of the participants. Several characteristics differed between men and women. Smoking status, alcohol habit, history of CVD, prevalence of antidiabetic medication, HGS, and serum T-B were higher in men than in women. HDL-C, LDL-C, prevalence of antidyslipidemic medication, and HOMA-IR were higher in women than in men. There were no inter-group differences regarding BMI, exercise habit, TG, and HbA1c.

Simple relationships between variables and serum T-B

Table 2 shows the relationship between serum T-B and various confounding factors. In men, LDL-C and HGS correlated positively with serum T-B, while smoking status, prevalence of antidyslipidemic medication, and HbA1c correlated negatively with serum T-B. In women, HDL-C and HGS correlated positively with serum T-B, while HbA1c and HOMA-IR correlated negatively with serum T-B. Fig. 1 shows the correlation between HGS and serum T-B. The correlation coefficient between HGS and serum T-B were significant in both men (r = 0.156, *p* = 0.023) and women (r = 0.173, *p* = 0.003).

Table 1. Characteristics of subjects by sex.

Characteristics	Men N = 214	Women N = 302	*P*-value*
Age (years)	71 ± 8	71 ± 7	0.946
Body mass index (kg/m^2)	23.0 ± 2.8	22.5 ± 3.2	0.078
Smoking status[†] (%)	38.8/46.3/4.2/10.7	97.7/1.0/0.7/0.7	**< 0.001**
Alcohol habit[‡] (%)	22.4/21.5/22.4/33.6	71.2/22.2/4.6/2.0	**< 0.001**
Exercise habit (%)	37.9	39.7	0.714
History of CVD (%)	12.1	6.3	**0.026**
Triglycerides (mg/dl)	88 (66 - 127)	85 (65 - 116)	0.097
HDL cholesterol (mg/dl)	62 ± 15	68 ± 17	**< 0.001**
LDL cholesterol (mg/dl)	114 ± 28	123 ± 29	**0.001**
Antidyslipidemic medication (%)	14.5	30.8	**< 0.001**
HbA1c (%)	5.7 (5.4 - 6.0)	5.7 (5.5 - 6.0)	0.406
Antidiabetic medication (%)	14.0	6.3	**0.004**
HOMA-IR	1.15 (0.80 - 1.83)	1.42 (0.96 - 2.26)	**0.003**
Handgrip strength (kg)	33.5 (27.8 - 39.0)	21.2 (18.5 - 23.7)	**< 0.001**
Serum bilirubin (mg/dL)	0.7 (0.5 - 0.9)	0.6 (0.5 - 0.8)	**< 0.001**

CVD, cardiovascular disease; HDL, high-density lipoprotein; LDL, low-density lipoprotein; Hb, hemoglobin; HOMA-IR, homeostatic model assessment for insulin resistance.
[†]smoking status was defined as the number of packs of cigarette smoked per day multiplied by the number of years (pack-year), and the participants were classified as never smokers, past smokers, light smokers (< 20 pack-year) and heavy smokers (≥ 20 pack-year).
[‡]Alcohol habit was measured using the Japanese liquor unit in which a unit corresponds to 23 g of ethanol, and the participants were classified as never drinkers, occasional drinkers, daily light drinker (< 2 unit/day), and daily heavy drinkers (≥ 2 unit/day).
Data are means ± standard deviation. Data for serum bilirubin, triglycerides, HbA1c, HOMA-IR, and handgrip strength were skewed and are presented as median (interquartile range) values, and were log-transformed for analysis.
*Student's t-test was used for the continuous data and χ^2 test for the categorical data. Numbers in bold indicate significance ($p < 0.05$).

Multivariate relationships between variables and serum T-B

As shown in Table 3, multiple linear regression analysis showed that in men, smoking status ($\beta = -0.147$), exercise habit ($\beta = 0.138$), LDL-C ($\beta = -0.146$), HbA1c ($\beta = -0.198$), and HGS ($\beta = 0.173$) were significantly and independently associated with serum T-B, and in women, smoking status ($\beta = -0.116$), HDL-C ($\beta = 0.159$), HbA1c ($\beta = -0.161$), and HGS ($\beta = 0.159$) were also significantly and independently associated with serum T-B.

Mean (95% CI) of serum T-B of the subjects categorized by handgrip strength

Table 4 presents the levels of HGS after adjustment for all confounding factors in Model 2. Serum T-B levels were significantly lower in subjects with low HGS levels in both men and women.

Discussion

To determine any possible contribution of HGS to serum T-B, we studied the relationship between confounding factors including HGS and serum T-B in this cross-sectional, population-based study. This study showed that HGS was independently and positively related to serum T-B. Increased HGS occurred in parallel with increase in serum T-B in both sexes, and HGS quartiles were significantly and positively associated with serum T-B, independent of other confounding factors in both sexes. To our knowledge, this is the first study to demonstrate a positive relationship between HGS and serum T-B among Japanese community-dwelling persons.

The precise mechanisms that lead to an increased serum T-B level in individuals with elevated HGS are not

Table 2. A simple relationship between variables including handgrip strength and serum total bilirubin within each sex.

Characteristics	Men N = 214 r (P-value)	Women N = 302 r (P-value)
Age	−0.080 (0.244)	−0.052 (0.372)
Body mass index	0.086 (0.212)	0.005 (0.938)
Smoking status	**−0.134 (0.049)**	−0.054 (0.347)
Alcohol habit	0.120 (0.079)	−0.033 (0.571)
Exercise habit	0.109 (0.113)	−0.037 (0.522)
History of CVD	−0.088 (0.201)	−0.074 (0.200)
Triglycerides	−0.057 (0.407)	−0.085 (0.143)
HDL cholesterol	0.104 (0.128)	**0.170 (0.003)**
LDL cholesterol	**0.135 (0.049)**	0.106 (0.066)
Antidyslipidemic medication	**−0.135 (0.048)**	−0.054 (0.350)
HbA1c	**−0.172 (0.012)**	**−0.165 (0.004)**
Antidiabetic medication	−0.027 (0.697)	−0.110 (0.056)
HOMA-IR	−0.092 (0.182)	**−0.167 (0.004)**
Handgrip strength	**0.156 (0.023)**	**0.173 (0.003)**

r, Pearson's correlation coefficient.
Data for serum bilirubin, triglycerides, HbA1c, HOMA-IR, and handgrip strength were skewed and log-transformed for analysis. Numbers in bold indicate significance ($p < 0.05$).

Fig. 1. Relationship between handgrip strength and serum total bilirubin within each sex.

completely understood. It was demonstrated that oxidative stress is a major modulator of skeletal muscle regeneration after injury, and balance between reactive oxygen species (ROS) production, antioxidant enzymes expression and activity plays an important role in maintaining muscle homeostasis (Kozakowska et al. 2015). Numerous nonenzymatic antioxidants [e.g., vitamins E and C, beta-carotene, glutathione (GSH), uric acid, ubiquinone, and bilirubin.] exist in cells and relates to skeletal muscle exercise (Powers and Jackson 2008). Baranano et al. (2002) and Sedlak et al. (2009) showed that bilirubin is a potent antioxidant that protects cells from a 10,000-fold excess of oxidants through rapid regeneration of bilirubin by biliverdin reductase, and that bilirubin is an important physiologic antioxidant as it inhibits both lipid and protein oxidation. In addition, bilirubin attenuates vascular endothelial activation and dysfunction in response to proinflammatory stress (Kawamura et al. 2005). Moreover, oxidative stress and inflammation contribute to that as the most common causes of atherosclerosis (Fan and Watanabe 2003). Furthermore, serum T-B correlated with several confounding risk factors for CVD, such as sex, age, smoking, alcohol, blood pressure, HDL-C, TG, LDL-C, diabetes, and obesity, and correlated directly with HDL-C (Kunutsor et al. 2015). Also in our study, serum T-B in men correlated with smoking sta-
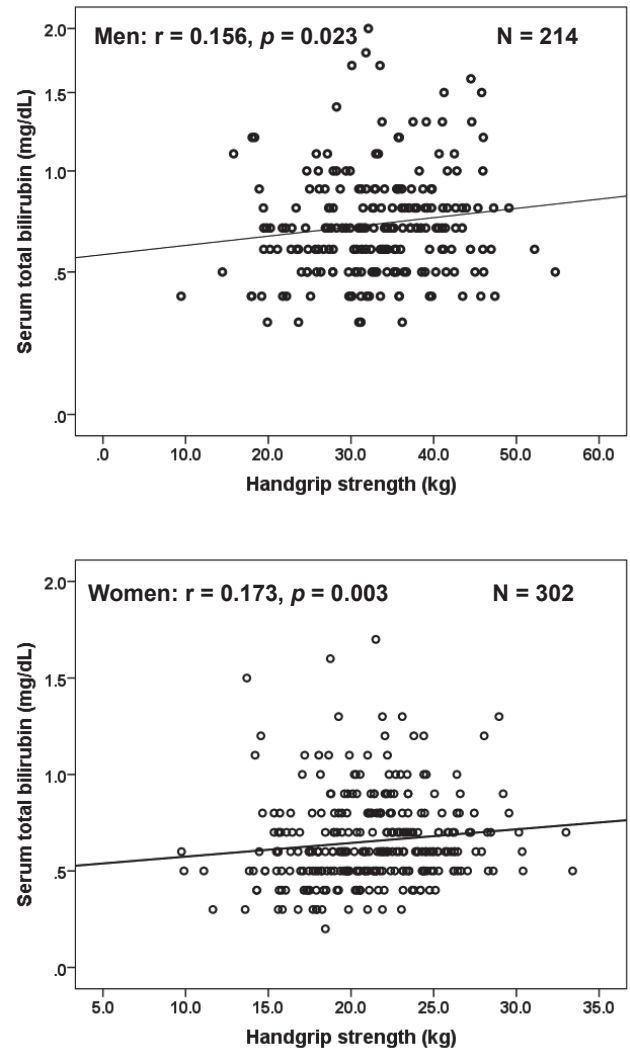
tus, exercise habit, LDL-C, and HbA1c, and in women with smoking status, HDL-C, and HbA1c. These contributions may appear to allow bilirubin to inhibit multiple steps in atherogenesis. In fact, previous epidemiological studies have demonstrated that serum T-B was negatively correlated with the presence of CVD (Perlstein et al. 2008; McArdle et al. 2012; Kawamoto et al. 2014; Kunutsor et al. 2015). These results support the finding that enhanced skeletal muscle and increased serum T-B play some role through their anti-atherogenetic actions.

There are some limitations to this study. Firstly, based on its cross-sectional study design, the present findings are inherently limited in the ability to eliminate causal relationships between confounding factors including T-B and HGS evaluated by only two right and left observations. Secondly, the possible effects of underlying diseases (e.g., liver disease, gallstones, and excessive red cell destruction) and medications used for hypertension and dyslipidemia on the present findings could not be eliminated. In our study, participants with serum T-B > 2.0 mg/dL or ALT ≥ 100 IU/

Table 3. Multiple linear regression analysis of variables including handgrip strength for serum total bilirubin within each sex.

	Men N = 214		Women N = 302	
	Multiple linear regression analysis		Multiple linear regression analysis	
	Model 1	Model 2	Model 1	Model 2
Characteristics	β(*P*-value)	β(*P*-value)	β(*P*-value)	β(*P*-value)
Age	−0.030 (0.735)	-------	0.063 (0.333)	-------
Body mass index	**0.174 (0.033)**	-------	0.117 (0.068)	-------
Smoking status	**−0.148 (0.033)**	**−0.147 (0.029)**	−0.095 (0.119)	**−0.116 (0.042)**
Alcohol habit	0.103 (0.151)	-------	−0.073 (0.242)	-------
Exercise habit	**0.155 (0.024)**	**0.138 (0.037)**	−0.021 (0.702)	-------
History of CVD	−0.062 (0.362)	-------	−0.044 (0.445)	-------
Triglycerides	−0.050 (0.531)	-------	0.023 (0.733)	-------
HDL cholesterol	0.065 (0.376)	-------	**0.208 (0.003)**	**0.159 (0.005)**
LDL cholesterol	0.125 (0.081)	**0.146 (0.030)**	0.079 (0.202)	-------
Antidyslipidemic medication	−0.074 (0.308)	-------	0.029 (0.645)	-------
HbA1c	**−0.230 (0.003)**	**−0.198 (0.003)**	**−0.142 (0.041)**	**−0.161 (0.004)**
Antidiabetic medication	0.149 (0.060)	-------	−0.010 (0.882)	-------
HOMA-IR	−0.115 (0.182)	-------	−0.121 (0.067)	-------
Handgrip strength	0.127 (0.139)	**0.173 (0.011)**	**0.169 (0.008)**	**0.159 (0.005)**
R^2	**0.174 (< 0.001)**	**0.115 (< 0.001)**	**0.123 (< 0.001)**	**0.087 (< 0.001)**

β, standardized coefficient; R^2, multiple coefficient of determination. Model 1, the forced entry method and Model 2, the forward stepwise method.

Data for serum bilirubin, triglycerides, HbA1c, HOMA-IR, and handgrip strength were skewed and log-transformed for analysis. Numbers in bold indicate significance ($p < 0.05$).

Table 4. Mean (95% CI) of serum total bilirubin of the subjects categorized by handgrip strength within each sex.

| | Men   N = 214 | | | Women   N = 302 | | |
| | | Non-adjusted | Multivariate-adjusted | | Non-adjusted | Multivariate-adjusted |
Characteristics	N	mean (95% CI)	mean (95%CI)[§]	N	mean (95%CI)	mean (95%CI)[§]
Quartile - 1	52	0.64 (0.58 - 0.71)	0.64 (0.58 - 0.71)	77	0.54 (0.51 - 0.59)	0.55 (0.51 - 0.59)
Quartile - 2	55	0.70 (0.64 - 0.77)	0.69 (0.63 - 0.76)	76	0.62 (0.58 - 0.67) b	0.62 (0.58 - 0.67) b
Quartile - 3	54	0.69 (0.63 - 0.76)	0.69 (0.63 - 0.76)	77	0.67 (0.63 - 0.73) d	0.67 (0.62 - 0.72) d
Quartile - 4	53	0.74 (0.67 - 0.82) a	0.76 (0.68 - 0.83) a	72	0.64 (0.59 - 0.69) c	0.63 (0.59 - 0.68) c

CI, confidence interval.

[§]Adjusted for all confounding factors in model 2 of Table 4. Subjects were divided into four groups based on quartile of handgrip strength within each sex (men, Quartile-1, 9.45-27.79; Quartile-2, 27.80-33.50; Quartile-3, 33.51-38.99; Quartile-4, 39.00-54.7 kg and women, Quartile-1, 9.75-18.49; Quartile-2, 18.50-21.30; Quartile-3, 21.31-23.80; Quartile-4, 23.81-33.40 kg). a, $p < 0.05$; b, $p < 0.02$; c, $p < 0.01$; and d, $p < 0.001$ versus Quartile-1.

L or GGT ≥ 100 IU/L were excluded, but those with Gilbert's syndrome may have been included in higher T-B group (1.00-2.00 mg/dL). Thirdly, in this study our model could account only for 8.7% to 17.4% of the variance in serum T-B. Success in the secondary prevention of obesity, hypertension, dyslipidemia and diabetes may be in regulating confounding factors. Therefore we must acknowledge the limitation of the demographics and referral source.

In conclusion, the present study showed that HGS is strongly associated with serum T-B in both men and women. The underlying mechanism behind this relationship still remains unclear, but it seems to be independent of confounding factors such as age, BMI, smoking status, drinking status, exercise habit, lipids, insulin resistance, and medication. Further prospective studies are needed to explore potential mechanisms for the association.

Author Contributions

Conceived and designed the experiments: RK. Performed the experiments: RK and DN. Analyzed the data: RK. Contrib-

uted reagents/materials/analysis tools: RK, DN, and TK. Wrote the paper: RK. Critical review of the manuscript: RK, DN, and TK.

Conflict of Interest

The authors declare no conflict of interest.

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