

Retinol Binding Protein-4 Elevation Is Associated with Serum Thyroid-Stimulating Hormone Level Independently of Obesity in Elderly Subjects with Normal Glucose Tolerance

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Context: Elevated levels of retinol binding protein-4 (RBP4) are positively correlated with insulin resistance, obesity, diabetes mellitus, and cardiovascular disease (CVD). Subclinical hypothyroidism (SCH) has also been associated with CVD; however, the factors linking SCH to CVD are not clear.

Objective: The objective of the study was to evaluate risk factors for CVD in elderly patients grouped according to thyroid function.

Design: 217 subjects (≥ 65 yr old) were randomly selected from a population and allocated to a euthyroid group ($n = 177$) and an SCH group ($n = 40$) on the basis of plasma concentrations of TSH and free T_4 . We included subjects with normal glucose tolerance by a 75-g oral glucose tolerance test and subjects with impaired fasting glucose. We measured anthropometric parameters, levels of fasting glucose and insulin, hemoglobin A_{1c} , adiponectin, RBP4, lipid profiles, total body fat content, and the area of sc and visceral fat.

Results: The SCH group had higher RBP4 levels than the euthyroid group, irrespective of body mass index and fat content. Subcutaneous and visceral fat areas and total body fat percentage did not differ between groups and were not correlated with RBP4 level. Other CVD risk factors did not differ between groups. RBP4 level was positively correlated with TSH level ($r = 0.241$, $P = 0.001$) after adjustment for age, sex, and body mass index.

Conclusions: Plasma RBP4 levels were associated with SCH independent of obesity in elderly subjects with normal glucose tolerance, indicating that RBP4 level could be used as an index of CVD risk in SCH. (*J Clin Endocrinol Metab* 93: 2313–2318, 2008)

Retinol binding protein-4 (RBP4) was recently identified as a novel adipocytokine that is expressed and secreted by adipose tissue and the liver (1). RBP4 expression is elevated in the adipose tissue of adipocyte-specific GLUT4 knockout mice and is substantially elevated in obese and insulin-resistant humans. Insulin resistance in mice can be induced by RBP4 overexpression or by injection of recombinant RBP4; RBP4 knockout mice are insulin sensitive (1–3). Although several clinical studies have

shown positive correlations between RBP4 level and insulin resistance (4–10), not all reports are consistent (11). Serum RBP4 level is also correlated with visceral adiposity (12), body mass index (BMI) (3), liver fat content (13), incipient nephropathy in type 2 diabetes (14), and dyslipidemia (15), which suggests that an elevated RBP4 level may be associated with the cardiovascular risk factors that accompany insulin resistance in metabolic syndrome (3).

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Abbreviations: BMI, Body mass index; CVD, cardiovascular disease; Eu, euthyroidism; ft_4 , free T_4 ; HbA_{1c} , hemoglobin A_{1c} ; HC, hip circumference; HDL, high-density lipoprotein; HOMA-IR, homeostasis model assessment-insulin resistance; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; LBM, lean body mass; NGT, normal glucose tolerance; OGTT, oral glucose tolerance test; RBP4, retinol binding protein-4; SBP, systolic blood pressure; SCH, subclinical hypothyroidism; SFA, sc fat area; TG, triglyceride; VFA, visceral fat area; WC, waist circumference; WHR, waist to hip ratio.

Cardiovascular disease (CVD) is a major cause of morbidity and mortality, and it is well known that thyroid dysfunction, especially hypothyroidism, is associated with increased cardiovascular risk (16). The prevalence of subclinical hypothyroidism (SCH) is high among the elderly (17), and cardiometabolic risk factors are particularly important in older individuals with abnormal thyroid function (18). It has been suggested by some that SCH is an independent risk factor for CVD (19–22), but other reports are conflicting (23–26).

There are no reports on the association of RBP4, an emerging surrogate marker for cardiometabolic risk, with SCH. Therefore, we compared several metabolic parameters, including plasma RBP4 concentrations, in euthyroid and subclinically hypothyroid elderly individuals who did not have diabetes or impaired glucose tolerance (IGT) to elucidate the effect of abnormal glucose metabolism on adipocytokines and CVD risk factors.

Subjects and Methods

Subjects

Between September 2005 and September 2006, 992 elderly individuals 65 yr old or older and living in Seongnam City, South Korea, were randomly selected. They were then contacted by letter and telephone and asked to participate in the Korean Longitudinal Study on Health and Aging. The study was designed as a population-based prospective cohort study of Korean elderly people. After exclusion of 420 respondents who had been treated as diabetes or dyslipidemia, 572 subjects were enrolled in this study. Respondents were classified into five groups on the basis of the results of a 75-g oral glucose tolerance test (OGTT) and the American Diabetes Association criteria (27). The groups were: normal glucose tolerance (NGT) (n = 163), impaired fasting glucose (IFG) only (n = 118, fasting glucose = 100–125 mg/dl), IGT only (n = 90, OGTT 2-h glucose = 140–199 mg/dl), both IFG and IGT (n = 95), and recently diagnosed with diabetes (n = 106, fasting glucose \geq 126 mg/dl or OGTT 2-h glucose \geq 200 mg/dl). We selected 281 subjects with NGT or IFG only to minimize the effect of glucose metabolism on RBP4 level. Of these 281 subjects, 217 elderly persons whose height and weight were available were finally selected (126 NGT and 91 IFG). Of the 217 subjects selected, 177 had normal thyroid function and 40 had SCH, as determined by a thyroid function test. For the thyroid function test, serum TSH and free T₄ (fT₄) concentrations were measured using an immunoradiometric assay (Abbott, North Chicago, IL). Euthyroidism (Eu) was defined as a normal level of TSH (0.4–4.1 μ U/liter) and fT₄ (0.7–1.8 ng/dl) in the absence of thyroid medication. SCH was defined as a TSH concentration greater than 4.1 μ U/liter and a normal fT₄ concentration. The Institutional Review Board of the Seoul National University Bundang Hospital approved the study protocol, and written informed consent was obtained from all subjects.

Questionnaires and anthropometric measurements

During the initial visit, all subjects completed a standardized questionnaire designed to evaluate their medical history. The questionnaire included questions about the history of diabetes mellitus, hypertension, and thyroid dysfunction. All participants attended a standardized clinical interview and underwent physical examination by three clinicians who were experts in geriatric research. All of the assessments were performed at the Seoul National University Bundang Hospital.

The respondents' height and weight were measured using an automatic height-weight scale while they wore light clothing. BMI was then calculated as the ratio between weight and height squared (kilograms per square meter). Blood pressure was measured twice on one occasion using a standard mercury sphygmomanometer after 10 min rest while the sub-

ject was in the sitting position. Waist circumference (WC) was measured at the narrowest point between the lower border of the rib cage and the iliac crest. Hip circumference (HC) was measured at the level of the symphysis pubis and the greatest gluteal protuberance. The waist to hip ratio (WHR) was then calculated by dividing WC by HC.

Measurement of body fat

Percentage body fat and lean body mass were measured using a tetrapolar bioelectrical impedance analyzer (Inbody 3.0; Biospace, Seoul, Korea). Bioelectrical impedance measures two parameters, fat and lean tissue, using empirically derived formulas, which have been validated and yield estimates that correlate with estimates obtained using underwater weighing (28). Abdominal adipose tissue (visceral fat area and sc fat area; expressed as square centimeters) were quantified using single computed tomography scans (Somatom Sensation 16; Siemens, Erlangen, Germany). A 5-mm computed tomography slice scan was acquired at the umbilical level to measure total abdominal and visceral fat area, and adipose tissue attenuation was then determined by measuring the mean value for all pixels within the range of –190 to –30 Hounsfield units.

Measurement of levels of glucose and insulin and lipid profiles

Plasma glucose concentration was measured using the glucose oxidase method and a YSI 2300 STAT glucose analyzer (Yellow Spring Instrument Co., Yellow Springs, OH). Plasma insulin concentrations were measured using a RIA (Linco Research, Chesterfield, MO). The interassay coefficient of variation was 4%. This assay did not cross-react with human proinsulin (<0.2%). The degree of insulin resistance was then estimated using the homeostasis model assessment method as described by Matthews *et al.* (29). The homeostasis model assessment for insulin resistance (HOMA-IR) was computed using the formula: fasting plasma glucose concentration (millimoles per liter) \times fasting serum insulin level (milliunits per liter)/22.5. Total cholesterol, triglycerides (TG), and high-density lipoprotein (HDL)-cholesterol concentrations were measured enzymatically using an autoanalyzer (Hitachi 747; Hitachi, Ltd., Tokyo, Japan). The level of low-density lipoprotein-cholesterol was estimated using the following formula (30): total cholesterol level – HDL-cholesterol level – (TG level/5).

Measurement of plasma RBP4 and adiponectin levels

Plasma RBP4 levels were measured using an ELISA (AdipoGen, Inc., College of Life Science and Biotechnology, Korea University, Seoul, Korea) as described in our previous study (31). The ELISA system had an intraassay coefficient of variation of 4–8% and an interassay coefficient of variation of 5–10% (31). Adiponectin levels were measured using an ELISA kit according to the manufacturer's instructions (AdipoGen).

Statistical analysis

All continuous variables that had a normal distribution are expressed as the mean \pm SD. The baseline clinical characteristics of the study subjects were compared using a *t* test or χ^2 test and binary logistic regression analysis as appropriate. Mean RBP4 levels according to the age group were compared using one-way ANOVA. Bivariate and partial correlation analyses were used to determine the correlation between levels of RBP4 and TSH. All statistical analyses were performed using SPSS software (SPSS Inc., Chicago, IL). *P* < 0.05 was considered statistically significant.

Results

Clinical and metabolic characteristics of subjects

There were no differences between subjects with Eu and those with SCH in age, sex, blood pressure, WC, HC, WHR, level of

fasting plasma glucose, 2-h postload glucose, and hemoglobin A_{1c} (HbA_{1c}), indices of insulin resistance (fasting insulin level, HOMA-IR), or lipid profiles (Table 1). BMI, visceral fat area, sc fat area (SFA), and percentage body fat did not differ between the Eu and SCH groups. We also classed subjects into a group comprising individuals of normal weight (BMI < 23 kg/m²) and a group comprising overweight individuals (BMI ≥ 23 kg/m²) on the basis of the World Health Organization expert consultation for Asian populations (32). Among the 217 individuals, 111 (Eu: SCH = 86:25) were assigned to the normal weight group and 106 (Eu: SCH = 91:15) were assigned to the overweight group. Age, sex, anthropometric measurements, fasting insulin level, and HOMA-IR did not differ between the Eu and SCH groups, regardless of the degree of obesity (data not shown). However, TG level was greater for the SCH group than for the Eu group within the normal weight group (132.8 ± 63.3 vs. 104.3 ± 60.8 mg/dl, respectively; *P* = 0.015). Lipid profiles other than TG did not differ between the Eu and SCH groups.

Correlation of RBP4 concentration with metabolic and biochemical characteristics

RBP4 level was greater for the SCH group than the Eu group (Table 1) and was greater for SCH than Eu when individuals in both the normal weight (51.0 ± 27.7 vs. 77.8 ± 52.4 kg, *P* = 0.001) and overweight groups (54.2 ± 20.4 vs. 67.5 ± 20.8 kg, *P* = 0.022) were considered. In addition, RBP4 level was positively correlated with TSH level in combined Eu and SCH group and remained significant after adjustment for age, sex, and BMI (*r* = 0.241, *P* < 0.001, Fig. 1), SFA (*r* = 0.273, *P* = 0.001) or visceral fat area (VFA) (*r* = 0.263 *P* = 0.002). RBP4 level was

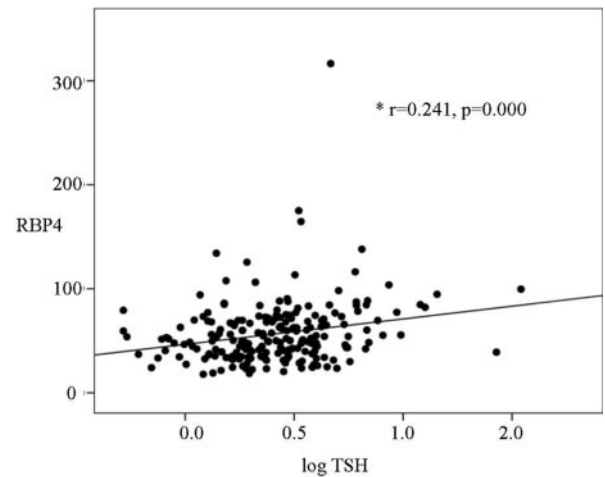


FIG. 1. Plasma RBP4 level was positively correlated with TSH level in elderly individuals with Eu and SCH after adjustment for age, sex, and BMI. Pearson correlation coefficient: *r* = 0.241, *P* < 0.001.

also correlated with adiponectin level (*r* = -0.286, *P* = 0.000) and systolic blood pressure (SBP) (*r* = 0.209, *P* = 0.002) after adjustment for age and sex. However, RBP4 level was not correlated with BMI, percentage body fat, SFA, or VFA (Fig. 2).

Fasting insulin and HOMA-IR were not correlated with RBP4 level (data not shown). Although SBP was significantly correlated with RBP4 level, diastolic blood pressure was not. There was also no correlation between RBP4 level and obesity parameters (percentage body fat, VFA, SFA, total fat area, WC, HC). Furthermore, lipid profiles were not correlated with RBP4 level (data not shown).

TABLE 1. Anthropometric and metabolic characteristics of elderly individuals with Eu or SCH

	Eu (n = 177)	SCH (n = 40)	<i>P</i>	Adjusted <i>P</i> ^a
Age (yr)	77.2 ± 8.9	78.6 ± 10.0	0.389	
Sex (male:female)	89:88	17:23	0.388	
SBP (mm Hg)	129.9 ± 16.3	135.4 ± 21.1	0.127	0.086
Diastolic blood pressure (mm Hg)	82.0 ± 10.2	84.0 ± 12.3	0.344	0.249
BMI (kg/m ²)	22.7 ± 3.7	22.4 ± 2.7	0.604	0.792
WC (cm)	85.4 ± 9.0	84.9 ± 10.0	0.757	0.735
WHR	0.93 ± 0.09	0.92 ± 0.09	0.631	0.552
VFA (cm ²)	100.8 ± 61.0	95.1 ± 50.7	0.676	0.886
SCA (cm ²)	141.5 ± 72.6	153.1 ± 76.6	0.490	0.584
Total fat area (cm ²)	242.3 ± 120.4	248.2 ± 116.8	0.830	0.808
Body fat (%)	26.6 ± 7.8	26.7 ± 6.7	0.944	0.614
LBM (kg)	37.9 ± 8.1	37.6 ± 7.6	0.829	0.285
Fasting plasma glucose (mg/dl)	98.2 ± 10.6	95.2 ± 10.6	0.107	0.161
HbA _{1c} (%)	5.65 ± 0.37	5.62 ± 0.35	0.558	0.556
Fasting insulin (μU/ml)	4.38 ± 2.58	4.39 ± 2.99	0.840	0.825
HOMA-IR	1.08 ± 0.74	1.05 ± 0.78	0.828	0.599
Total cholesterol (mg/dl)	200.4 ± 37.1	201.7 ± 34.6	0.842	0.914
TG (mg/dl)	123.5 ± 80.5	140.2 ± 77.6	0.124	0.116
HDL-cholesterol (mg/dl)	62.2 ± 16.4	57.3 ± 14.7	0.085	0.098
LDL-cholesterol (mg/dl)	114.7 ± 32.0	117.1 ± 31.0	0.667	0.806
TSH (μU/ml)	2.29 ± 0.93	7.67 ± 5.93	0.000	0.953
FT4 (ng/dl)	1.23 ± 0.28	1.17 ± 0.28	0.247	0.342
Adiponectin (μg/ml)	11.1 ± 7.2	10.8 ± 7.2	0.768	0.498
RBP4 (μg/ml)	52.7 ± 24.2	74.0 ± 47.0	0.000	0.000

^a *P* adjusted for age, sex, and BMI. Values are mean ± standard deviation.

LDL, Low-density lipoprotein.

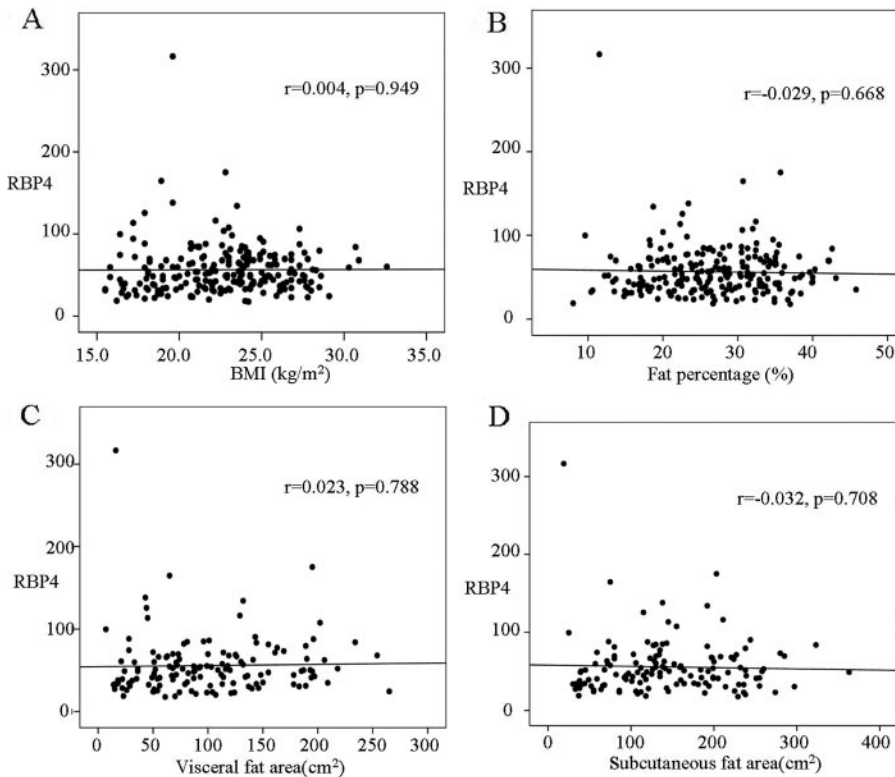


FIG. 2. Plasma RBP4 level was not correlated with BMI (A), fat percentage (B), VFA (C), or SFA (D).

Correlation between age and RBP4 level

Because we enrolled only people over 65 yr of age, we investigated the association between age and RBP4 level. RBP4 level did not differ between the age groups ($P = 0.20$), which included a very old group (over 85 yr), or correlate with age ($r = 0.025$, $P = 0.715$).

Correlation between adiponectin level and metabolic and biochemical variables

Although the level of adiponectin was not correlated with TSH level ($r = 0.109$, $P = 0.109$), age, level of HbA_{1c}, TG, and HDL-cholesterol, VFA, SFA, WC, HC, lean body mass (LBM), BMI, fasting insulin level, and HOMA-IR were all significantly correlated with adiponectin level. However, only HDL-cholesterol level ($r = 0.349$, $P < 0.001$), VFA ($r = -0.332$, $P < 0.001$), and TFA ($r = -0.272$, $P = 0.002$) were significantly associated with adiponectin after the data were adjusted for age, sex, and BMI.

Discussion

This study indicated that subjects with SCH had significantly greater plasma RBP4 levels than those with normal thyroid function and that TSH level was correlated with RBP4 level in elderly individuals with normal glucose tolerance irrespective of the degree of obesity or the amount of body fat. It has been reported that RBP4 level is affected by glucose intolerance, obesity, adiposity, and age. Of these factors, glucose intolerance has consistently been associated with RBP4 levels (4–10). Therefore, to

exclude the effects of glucose intolerance on RBP4, we analyzed data only from elderly subjects who had NGT or IFG only. Plasma RBP4 level was significantly correlated with TSH level, regardless of the level of fasting glucose, HbA_{1c}, fasting insulin, or HOMA-IR, even though glucose tolerance was normal.

Although obesity is considered to be associated with overt hypothyroidism (33), few studies have been conducted on SCH. A recent study showed no relationship between SCH and morbid obesity (mean BMI of subjects was over 52.52 kg/m²) (34). Although the prevalence of SCH is greater in obese individuals, TSH level has only been associated with insulin resistance and not with BMI, fat mass, or LBM (34, 35). This suggests that obesity or body fat *per se* could not explain the metabolic abnormalities observed in individuals with SCH. In our study, no significant correlation was observed between TSH level and BMI or fat percentage. When we classed our subjects into two groups

using a BMI cutoff value of 23 kg/m², plasma RBP4 level was significantly and positively correlated with TSH level in both the overweight and normal weight groups. Thus, altered adipocytokine metabolism could be related to metabolic disorders in SCH.

Several previous studies have shown that RBP4 concentrations are elevated in obese patients (12, 36, 37). However, in our study plasma RBP4 level did not correlate with BMI or the amount of visceral fat amount measured using computed tomography. Gavi *et al.* (7) also reported no correlation between RBP4 level and BMI. Their study included 46 nonobese elderly individuals aged 60–83 yr who did not have diabetes. Taken together, factors other than BMI or fat mass may affect circulating RBP4 levels, even though RBP4 is produced by adipocytes.

TG level was significantly greater in the SCH group than the Eu group within the normal weight group; however, this was not the case within the overweight group. Although an association between lipid profile and BMI has been established, TG level is associated with BMI in men but not women (8, 38). In our study, the normal-weight group included more male subjects than did the overweight group, which may explain why we observed a positive correlation between TG level and BMI only in the normal-weight group. Some reports have shown that RBP4 affects fatty acid metabolism (8, 39), but we did not detect a significant correlation between TG and RBP4 levels in our study.

This study was conducted on individuals aged 65 yr and older. In this group, RBP4 level was not affected by age, even in the very old age group (>85 yr). Although several studies have been conducted to elucidate the relationship of RBP4 with age (3, 5–8, 36), only two have demonstrated a positive correlation with

age (7, 8). It is possible that the level of RBP4 in our elderly subjects was greater than that of young adults. However, within the elderly group, an association between RBP4 level and age was not evident.

We found that plasma adiponectin level was negatively correlated with plasma RBP4 level in elderly subjects with euthyroidism or SCH. It is well known that adiponectin has a counterregulatory role to that of RBP4. Although several studies have evaluated the correlation between adiponectin and RBP4 (12) or other metabolic parameters (13, 40, 41), the mechanism by which these adipocytokines interact is unclear. Additionally, the role of adiponectin in hypothyroidism is controversial. Some authors have reported that adiponectin levels in patients with hypothyroidism were similar to those in patients with euthyroidism and that adiponectin levels did not change significantly during L-thyroxine treatment (42). Others reported a significant association between adiponectin and TSH level but did not adjust their results for other confounding factors (43). Although adiponectin level was negatively correlated with RBP4 level in our study, further study is required on the mechanism by which the reciprocal effects of those adipocytokines are exerted.

In summary, plasma RBP4 levels were positively correlated with TSH levels, regardless of indices of obesity in elderly individuals with NGT and IFG only. Plasma RBP4 elevation was the only difference between the Eu and SCH groups. However, the mechanism by which RBP4 levels are increased in subjects with SCH remains unknown, and it is unclear whether increased RBP4 levels are a cause or a result of SCH. Despite these uncertainties, it is possible that elevated RBP4 levels may be associated with increased cardiometabolic risk in SCH as well as insulin resistance, obesity, and type 2 diabetes.

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