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Influence of thyroid dysfunction on serum levels of angiopoietin-like protein 6

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ABSTRACT

Angiopoietin-like protein 6 (ANGPTL6) is a novel metabolic regulator that modulates energy expenditure as well as glucose and lipid metabolism. Thyroid hormone can induce metabolic changes that are similar to those induced by ANGPTL6. Herein, we investigated whether circulating ANGPTL6 levels change according to thyroid hormone status in humans. We measured the serum levels of ANGPTL6 and metabolic parameters in 150 drug-naïve subjects with overt hyperthyroid, subclinical hyperthyroid, euthyroid, subclinical hypothyroid, or overt hypothyroid status (n = 30 in each group). Serum ANGPTL6 levels were significantly higher in patients with overt hypothyroidism than in the other subjects. Women had significantly higher serum levels of ANGPTL6 than men. ANGPTL6 levels correlated positively with thyroid stimulating hormone (TSH), total cholesterol, aspartate aminotransferase, and alanine aminotransferase (ALT) and negatively with serum free thyroxine (T4) level. Multiple stepwise linear regression analysis revealed that sex, TSH, free T4, and ALT were independent predictors of serum ANGPTL6 levels. In summary, serum ANGPTL6 levels increased in patients with a hypothyroid status, and both TSH and free T4 levels are associated with ANGPLT6 levels, suggesting a possible association between thyroid function and ANGPTL6 levels. Whether the upregulated ANGPTL6 level in the hypothyroid status is primarily owing to a direct association or a compensatory mechanism remains to be determined

1. Introduction

Several adipocyte- and hepatocyte-derived factors, the so-called adiopokines and hepatokines, are a critical link between obesity and metabolic disease [1,2]. A novel hepatokine, angiopoietin-like protein 6 (ANGPTL6) has been introduced as a member of the ANGPTL family, whose members are circulating orphan peptides secreted by the liver [3,4]. It is also known as angiopoietin growth factor, which induces angiogenesis and skin cell proliferation, and

Abbreviations: ANGPTL6, angiopoietin-like protein 6; TSH, thyroid stimulating hormone; AST, aspartate aminotransferase; ALT, alanine aminotransferase; T4, thyroxine; BMI, body mass index; LDL, low-density lipoprotein.

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thereby wound healing [3,5]. It was recently shown that ANGPTL6 is involved in the development of obesity and its related insulin resistance, and ANGPTL6 deficiency led to reduced energy expenditure in a mouse model [6]. In accordance with these findings, the mice with transgenic ANGPTL6 overexpression were lean and insulin sensitive and they showed increased energy expenditure and resistance to high fat diet-induced obesity, insulin resistance, and non-adipose tissue steatosis [6]. However, in contrast to the rodent data, human data showed elevated ANGPLT6 levels in patients with metabolic syndrome, preeclampsia, polycystic ovary syndrome, or type 2 diabetes mellitus [7–12]. This paradoxical elevation has been understood as ANGPTL6 resistance or a compensatory mechanism [9,10,12].

Thyroid hormone has an important role in different physiological processes such as development, growth, and metabolic control [13]. It has been recognized for more than 100 years that thyroid hormone increases obligatory thermogenesis [14], although the mechanisms involved are poorly understood. In fact, hypothyroid animals and patients typically experience a 20%–30% reduction in the resting metabolic rate and are more likely to develop hypothermia, whereas treatment with thyroid hormone increases the oxygen consumption in most tissues [13,15].

It has been suggested that adipokines and hepatokines as well as thyroid hormones share some physiological actions such regulating energy expenditure or lipid and glucose metabolism [16,17]. Because both thyroid hormones and ANGPTL6 are important regulators of energy metabolism, it could be hypothesized that the thyroid hormone status influences ANGPTL6 regulation. However, there are no human studies about the correlation between serum ANGPTL6 levels and thyroid hormones to date. Thus, here we evaluated the influence of the thyroid hormone status on serum ANGPTL6 levels in humans.

2. Material and Methods

2.1. Study Population

The medical records of consecutive subjects without previous history of thyroid disease who underwent a self-referred health checkup at the Seoul National University Bundang Hospital (SNUBH) Health Promotion Center between January 2008 and December 2009 were reviewed. We excluded subjects who presented with any known chronic disease requiring treatment, such as diabetes mellitus or chronic liver disease. We ultimately included 150 subjects (52 men, 98 women; mean age, 43.6 ± 10.0 years). The patients were then divided into overt hyperthyroid, subclinical hyperthyroid, euthyroid, subclinical hypothyroid, and overt hypothyroid groups (n = 30 each). The study protocol was approved by the Institutional Review Board of SNUBH (IRB No. B-0801-053-015).

2.2. Anthropometric and Biochemical Measurements

The height and weight were measured to the nearest 0.1 cm and 0.1 kg, respectively, and the body mass index (BMI) was calculated as weight divided by height squared (kg/m^2) at the time of blood sampling. A venous blood sample was taken after a minimum 12-h fast. Serum thyroid-stimulating hormone (TSH) and free thyroxine (T4) were measured by using immunoradiometry with commercial kits (TSH, CIS Bio International, Gif-sur-Yvette, France; free T4, DiaSorin, Saluggia, Italy). Euthyroidism was defined as normal levels of TSH (range, 0.4–4.5 mIU/L) and free T4 (range, 0.7–1.5 ng/dL). Overt hyperthyroidism was defined as a TSH level < 0.4 mIU/L and a free T4 level > 1.5 ng/dL; subclinical hyperthyroidism was defined as a TSH level < 0.4 mIU/L and a free T4 level within the normal range. Overt hypothyroidism was defined as a TSH level > 4.5 mIU/L and a free T4 level < 0.7 ng/dL; subclinical hypothyroidism was defined as a TSH level > 4.5 mIU/L and a free T4 level within the normal range.

The levels of glucose, total cholesterol, triglycerides, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein cholesterol, aspartate aminotransferase (AST), and alanine aminotransferase (ALT) were measured using the Toshiba 200FR Neo Chemistry autoanalyzer (Toshiba Medical Systems, Tokyo, Japan).

Serum ANGPTL6 levels were measured by using a Luminex® Multiplex assay using MILLIPLEX MAP Human Liver Protein Magnetic Bead Panel (Millipore, MO, USA). The system had an inter-assay coefficient of variation (CV) of 7.5%–18.4% and an intra-assay CV of 1.5%–3.3%.

2.3. Statistical Analysis

All normally distributed continuous variables are expressed as means ± SD, and variables with a skewed distribution are expressed as median and range. Variables with skewed distribution were log-transformed for the statistical analysis. Baseline characteristics were compared between groups using analysis of variance (ANOVA). To evaluate the differences in the levels of ANGPTL6 in various states of thyroid function, ANOVA with Bonferroni's *post hoc* analysis was applied. Pearson's correlation analysis identified significant correlations between ANGPTL6 and various metabolic parameters. Stepwise linear regression analysis was used to identify independent predictors of ANGPTL6 levels. Statistical analyses were performed using SPSS for Windows version 16.0. (SPSS, Chicago, IL, USA). Values of $P < 0.05$ were considered significant.

3. Results

3.1. Subjects' Clinical Characteristics and Serum ANGPTL6 Levels

The subjects' clinical characteristics are summarized in Table 1. The mean age did not differ among groups. The female to male ratio was higher in the overt hypothyroid subjects than in the others ($P = 0.003$). The mean BMI was comparable in all five groups. However, the lipid profiles and serum level of AST and ALT differed significantly among the groups: serum levels of total cholesterol, LDL-cholesterol, triglycerides, and AST were significantly higher in the overt hypothyroid subjects than in the other subjects (Table 1). The significance remained similar after the adjustment for sex (data not shown).