Plasma FGF21 levels are increased in patients with hypothyroidism independently of lipid profile

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Abstract. Thyroid hormone is a potent regulator of metabolic and energy homeostasis implicated in various metabolic diseases. Fibroblast growth factor 21 (FGF21) is a systemic metabolic regulator known to modulate various biological functions similar to the actions of thyroid hormone. We investigated the differences in plasma FGF21 concentrations in patients with varying thyroid function. Ninety drug-naïve subjects who underwent thyroid evaluation at Seoul National University Bundang Hospital were enrolled and classified into euthyroid, subclinical hypothyroid, and overtly hypothyroid groups. Biochemical markers and plasma FGF21 levels were measured and analyzed. The mean age of the subjects was 42.6 ± 9.1 years. The mean body mass index (BMI), waist circumference, and fasting glucose concentrations were similar between groups. Overtly hypothyroid subjects exhibited significantly higher concentrations of total cholesterol, triglyceride, and LDL-cholesterol than the other groups (p<0.01). Mean plasma FGF21 concentrations in euthyroid, subclinical hypothyroid and overtly hypothyroid groups were 43.2 ± 39.2 pg/mL, 63.6 ± 73.6 pg/mL, and 101.5 ± 74.9 pg/mL, respectively (p<0.01 between groups). Plasma FGF21 concentrations remained significantly higher in overtly hypothyroid subjects after adjusting for serum triglyceride concentrations (p<0.005). Multivariate analysis revealed a significant positive linear relationship between serum TSH concentrations and plasma FGF21 concentrations (β = 0.192, p = 0.002) and a significant negative linear relationship between free T4 and plasma FGF21 concentrations (β = –0.382, p = 0.037) after adjusting for gender, BMI and serum concentrations of triglycerides and glucose. Plasma FGF21 levels were significantly increased in patients with hypothyroidism independently of BMI, or lipid or glucose metabolism.

Key words: Hypothyroidism, Subclinical hypothyroidism, FGF21, Adipocytokine, Hyperlipidemia
tor, and adipocytokines such as leptin, resistin, and adiponectin. Serum leptin level was found to be unaltered in patients with thyroid dysfunction [1-3], whereas adiponectin was found to be increased in those with hyperthyroidism but unchanged in those with hypothyroidism, indicating a relationship between adipocytokines and immune or inflammatory derangement [1, 3, 4]. The effect of thyroid dysfunction on resistin is conflicting, but this adipocytokine has been found to have a positive association with serum free thyroxine levels and a negative association with thyroid-stimulating hormone levels. Moreover, it showed a significant decrease after treatment for hyperthyroidism [1, 3].

In recent years, fibroblast growth factor 21 (FGF21) has emerged as a promising metabolic regulator with high potential for a drug target. FGF21 works in a hormone-like manner and has been found to be linked to thermogenesis [5, 6], hyperglycemia [7, 8], hyperlipidemia [9], obesity [10], hepatic steatosis [11, 12] and metabolic syndrome [13]. While its upstream and downstream signals are still under investigation in various studies, the similarities in metabolic actions between FGF21 and thyroid hormone suggest that the metabolic actions of these two potent regulators may be closely related. In fact, a recent study suggested that thyroid hormone might regulate the hepatic expression of FGF21 in a PPARα-dependent manner [14]. However, there have been no studies investigating the relationship between thyroid function and FGF21 in human subjects. In this study, we examined the relationship between FGF21 and thyroid hormone in a large population of patients with different thyroid function.

**Anthropometric and biochemical measurements**

Height and weight were measured to the nearest 0.1 cm and 0.1 kg, respectively, and BMI was calculated as weight divided by height squared (kg/m²) at the time of blood sampling. A venous blood sample was taken at an outpatient department after a minimum 12h fast. Serum thyroid-stimulating hormone (TSH) and free thyroxine (T4) were measured by immunoradiometry using commercial kits (TSH, DiaSorin S.p.A., Saluggia, Italy; free T4, BRAHMS, Hennigsdorf/Berlin, Germany). Euthyroidism was defined as having normal levels of TSH (range 0.4–4.0 mIU/L) and free T4 (range 0.78–1.94 ng/dL). Subclinical hypothyroidism was defined as a TSH concentration >4.0 mIU/L and a free T4 concentration within the normal range; overt hypothyroidism was defined as a TSH concentration > 4.0 mIU/L and a free T4 concentration< 0.78ng/dL.

The serum concentrations of glucose, total cholesterol, triglycerides, low-density lipoprotein (LDL)-cholesterol, high-density lipoprotein (HDL)-cholesterol, aspartate aminotransferase (AST), alanine aminotransferase (AST) and C-reactive protein (CRP) were measured using the Toshiba 200FR Neo chemistry autoanalyzer (Toshiba Medical Systems Co., Ltd, Tokyo, Japan).

Plasma FGF21 concentrations were measured by Luminex® Multiplex assay using MILLIPELSX MAP Human Liver Protein Magnetic Bead Panel. (Millipore, Missouri, USA) which had a sensitivity of 3 pg/mL, an inter-assay coefficient of variation (CV) of 7.5 % and an intra-assay CV of 1.5 %, an accuracy of 98.8%, and a standard curve range of 10 ng/mL to 10,000 ng/mL.

**Statistical analysis**

All data are presented as the mean ± standard deviation (SD) and were analyzed using SPSS for Windows version 18.0 (SPSS Inc., Chicago, IL, USA). In figure, the standard error bar was represented instead of SD. The demographic and laboratory characteristics of subjects were compared using analyses of variance and covariance between patients with different thyroid function. Linear regression analysis was performed between serum TSH and free T4 concentrations and plasma FGF21 concentrations; p <0.05 was considered significant.

**Results**

The baseline characteristics of the 90 subjects are shown according to thyroid function status in Table 1. The study subjects consisted of 33 men and 57 women.
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Furthermore, we subdivided the subjects into two groups according to triglyceride levels with a cutoff value of 150 mg/dL, and found that this linear relationship between plasma FGF21 and serum TSH persisted both in subjects without hypertriglyceridemia ($\beta = 0.391$, $p = 0.001$) and in subjects with hypertriglyceridemia ($\beta = 0.549$, $p = 0.015$) and the correlation was stronger in patients with hypertriglyceridemia. The relationship between plasma FGF21 and serum free T4 concentrations showed similar trends ($\beta = -0.297$, $p = 0.013$ and $\beta = -0.580$, $p = 0.009$) for subjects with or without hypertriglyceridemia.

The mean plasma FGF21 concentrations were $43.2 \pm 39.2$ pg/mL, $63.6 \pm 73.6$ pg/mL, and $101.5 \pm 74.9$ pg/mL in euthyroid, subclinical hypothyroid and overtly hypothyroid subjects, respectively, and there was no significant gender difference ($62.8 \pm 70.7$ pg/mL vs $72.8 \pm 67.1$ pg/mL for males and females respectively, $p = 0.256$). Post hoc analysis revealed that this difference was only significant in the overtly hypothyroid group, and it remained significant after adjusting for serum triglyceride concentrations as shown in Fig. 1. Correction of plasma FGF21 levels with serum levels of other subtypes of lipids such as total cholesterol, LDL-cholesterol, and HDL-cholesterol also had no effect on the association of FGF21 and thyroid hormones.

Linear regression analysis showed a significant positive relationship between serum TSH concentrations and plasma FGF21 concentrations ($\beta = 0.192$, $p = 0.002$) and a significant negative relationship between serum free T4 concentrations and plasma FGF21 concentrations (Fig. 2). Furthermore, we subdivided the subjects into two groups according to triglyceride levels with a cutoff value of 150 mg/dL, and found that this linear relationship between plasma FGF21 and serum TSH persisted both in subjects without hypertriglyceridemia ($\beta = 0.391$, $p = 0.001$) and in subjects with hypertriglyceridemia ($\beta = 0.549$, $p = 0.015$) and the correlation was stronger in patients with hypertriglyceridemia. The relationship between plasma FGF21 and serum free T4 concentrations showed similar trends ($\beta = -0.297$, $p = 0.013$ and $\beta = -0.580$, $p = 0.009$) for subjects with or without hypertriglyceridemia.
Discussion

In this cross-sectional study, we showed that the plasma FGF21 concentration was significantly elevated in subjects with overt hypothyroidism. Serum cholesterol, triglyceride and LDL-cholesterol concentrations were also significantly higher in the overtly hypothyroid group as expected. Plasma FGF21 concentrations remained significantly higher even after adjusting for lipid parameters, suggesting that the elevation of FGF21 in overtly hypothyroid subjects might be an effect of thyroid hormone on FGF21 metabolism independently of dyslipidemia induced by hypothyroidism.

FGF21 is an emerging metabolic regulator, and has been found to be most evidently linked to glucose and lipid metabolism in previous studies. In vivo and in vitro studies have shown that FGF21 acts as a modulator of insulin and glucose metabolism, reversing obesity and hypoglycemia and increasing energy expenditure in diet-induced obese mice [15, 16]. FGF21 also acts on thermogenesis by increasing the expression of involved genes in fat tissues in response to cold exposure [6]. Subsequent studies in humans showed that plasma FGF21 levels were significantly associated with BMI, waist circumference and markers of insulin resistance, and were elevated in subjects with metabolic syndrome as well as with fatty liver diseases, which suggest FGF21 resistance may play a role in the pathogenesis of these diseases [17, 18]. The exact mechanism by which FGF21 works has not yet been elucidated, but is known to involve activation by the PPARα pathway [14, 19, 20] and modulation of PPARγ and PGC-1α [6, 21, 22].

Thyroid hormone, on the other hand, is a well-established metabolic regulator with diverse actions in the liver, brain, kidney and other organs and tissues. Its well-known actions include increased energy expenditure via influencing carbohydrate, protein and lipid metabolism, which share great similarities with the metabolic actions of FGF21. Thyroid hormone has been shown to affect resting energy expenditure significantly [23], stimulate amino acid uptake in vitro [24] and alter glucose kinetics both in vitro and in vivo [25, 26]. The association between thyroid hormone and lipid metabolism is especially well characterized, and hypercholesterolemia with adverse lipoprotein profile is considered a major feature of patients with hypothyroidism.

While the metabolic pathways of both FGF21 and thyroid hormone remain complex and not fully elucidated, a recent study reported that mice treated with exogenous T3 showed a dose-dependent increase in hepatic FGF21 expression and a decrease in FGF21 expression in white adipose tissue [14]. This finding vanished in gene knockout mice for PPARα, an upstream signal of FGF21 expression, suggesting a PPARα-dependent regulation of FGF21 by thyroid hormone. On the other hand, our study examined changes in circulating FGF21 in patients exposed to chronic

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**Fig. 2** Relationships between plasma FGF21 concentrations and serum TSH concentrations (A) and free T4 concentrations (B) in all subjects (n = 90). Pearson’s correlation coefficients were as follows: R = 0.436, p<0.01 (A) and R = –0.396, p<0.01 (B). Dotted lines represent 95% confidence interval.
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Low levels of thyroid hormones in comparison with subjects with normal thyroid function and showed that circulating FGF21 levels were increased in hypothyroid patients. Because both FGF21 and thyroid hormone are associated with altered lipid metabolism, we corrected plasma FGF21 levels for serum triglyceride levels which have been repeatedly shown to be associated with plasma FGF21 concentration [7, 13, 18]. The differences in FGF21 levels between groups remained statistically significant, after correction of FGF21 levels for serum triglyceride levels, indicating that the differences in circulating FGF21 levels were not caused by the secondary effects of dyslipidemia.

The mechanism by which circulating FGF21 levels are increased in patients with hypothyroidism is not clear. One possible explanation is that the increase in circulating FGF21 levels might be a compensatory mechanism in response to altered metabolism by thyroid hormone. Unlike a previous study in which mice were exposed to a maximum of 6h of exogenous thyroid hormones [14], our subjects represent a long-standing exposure to altered thyroid hormone levels. This raises the possibility of other target molecules that might require the genomic actions of thyroid hormone to influence their synthesis. Although our results suggest the exclusion of an altered lipid profile as a possible mechanism, other metabolic changes such as changed basal metabolic rate [9, 16] or body fat accumulation [27], which have not been assessed in this study, may have a role in stimulating the synthesis of FGF21. Another hypothesis involves possible cross-talk between thyroid hormone receptors and PPAR. A recently published article showed that LXR, a closely related nuclear receptor of PPAR, FXR and RXR, represses FGF21 gene expression at the transcription level and that this may involve the presence of an LXR response element in the FGF21 promoter [28]; this feature may be shared in PPAR. Alternatively, the increased circulating FGF21 concentration might be a response to local regulation by thyroid hormone. Thyroid hormone synthesis is under the influence not only of many systemic factors but also of local factors, which include the actions of deiodinases that work to convert T4 to T3. Consequently, an increase in hepatic deiodinase activity promoting a local increase in T3 might account for increased FGF21 synthesis in the liver. This explanation is especially plausible in explaining why the increase in FGF21 expression was liver-specific in the previous study. Lastly, the elevation of circulating FGF21 in patients with hypothyroidism might arise from an altered balance between synthesis and clearance. Thyroid hormone promotes metabolism by increasing both processes, and an imbalance between synthesis and clearance accounts for dyslipidemia in patients with hypothyroidism. Although the mechanism of FGF synthesis and clearance is unclear, an altered balance in synthesis and clearance might account for the increased levels of circulating FGF21.

Since the mechanism by which plasma FGF21 concentration is elevated is unclear, it may be untimely to draw the implications of increased plasma FGF21 concentrations in hypothyroidism at this point. However, the elevation of FGF21 in subjects with hypothyroidism, independently of measurable parameters of altered metabolism, shows that the pathways of energy metabolism are intermingled at a more fundamental level, and gives us a new insight to how thyroid hormone works for which further research may focus on to better understand thyroid physiology. Also, this raises the possibility that FGF21 may serve as a more comprehensive target in those patients whose symptoms are not fully improved by thyroid hormone replacement alone or in those who have either unable to take thyroid hormones or thyroid hormone resistant. Hence, FGF21 may serve as a marker helpful in identifying undetected metabolic derangements other than already known regulators of the FGF21 for those in need of further assessment.

There are some limitations to our study. First, it was a cross-sectional study and changes in plasma FGF21 concentrations after acute or chronic replacement of thyroid hormone were not assessed. In this study, we have allotted subjects into groups according to thyroid function before randomly selecting 30 subjects for each group in order to compare plasma FGF21 levels more efficiently in a limited number of subjects. This method may have produced selection bias although selection after allotment was random. By intentionally allotting patients into groups, we were also limited in our methods for statistical analysis, because serum levels of TSH and free T4 were not normally distributed. Also, the scope of our study was limited to measuring biochemical profiles, lipid profiles and plasma FGF21 levels. Other factors such as thyroid autoantibodies, other adipocytokines, hormones, and gene expression in tissues, and assessment of metabolic rates and other parameters that might have affected altered FGF21
were not examined, leaving the mechanism by which the FGF21 level is elevated in patients with hypothyroidism unidentified. Nevertheless, we have shown that the circulating FGF21 level correlates with the levels of serum TSH and free T4 and that circulating FGF21 is increased in patients with hypothyroidism independently of lipid profile. More studies to confirm our findings in larger populations with varying thyroid function including hyperthyroidism are needed.

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Declaration of Conflicts of Interest

The authors declare no conflicts of interest.

References

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