

## RESEARCH

# Elevated serum neuregulin 4 levels in patients with hyperthyroidism

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## Abstract

**Objective:** Recent studies have shown that neuregulin 4 (Nrg4), a member of the epidermal growth factor (EGF) family of extracellular ligands, plays an important role in the prevention of obesity, insulin resistance and nonalcoholic fatty liver disease (NAFLD). Considering that thyroid hormone (TH) has profound effects on whole-body energy metabolism, we speculate that circulating Nrg4 levels might be altered in patients with hyperthyroidism.

**Design and methods:** A total of 129 hyperthyroid patients and 100 healthy subjects were recruited. Of them, 39 hyperthyroid patients received thionamide treatment for 3 months until euthyroidism. Serum Nrg4 levels were determined using the ELISA method. To further confirm the relationship between TH and Nrg4, C57BL/6 mice were treated with T<sub>3</sub> and quantitative real-time PCR was performed to detect Nrg4 gene expression.

**Results:** Serum Nrg4 levels were significantly elevated in hyperthyroid patients as compared with normal controls ( $3.84 \pm 1.63$  vs  $2.21 \pm 1.04$  ng/mL,  $P < 0.001$ ). After achieving euthyroidism by thionamide treatment, serum Nrg4 levels dropped markedly from  $3.57 \pm 1.26$  to  $1.94 \pm 0.72$  ng/ml ( $P < 0.001$ ). After adjustment for potential confounders, serum Nrg4 levels were independently associated with hyperthyroidism. The upregulation of Nrg4 expression in the livers and white adipose tissues by T<sub>3</sub> was further confirmed by animal and cell culture experiments.

**Conclusions:** Serum Nrg4 levels were increased in patients with hyperthyroidism. The liver and white adipose tissue might be primary sources contributing to elevated serum Nrg4 concentrations.

## Key Words

- ▶ thyroid
- ▶ metabolism

Endocrine Connections  
(2019) 8, 728–735

## Introduction

Thyroid hormone (TH) is required for both normal development and metabolism (1, 2), acting through its two receptor isoforms,  $\alpha$  and  $\beta$ . TR $\alpha$  is mainly expressed in

the skeletal muscle, adipose tissue and heart, whereas TR $\beta$  is mainly expressed in the liver (1, 2). Hyperthyroidism, a state of excessive TH, is characterized by increased resting

energy expenditure, weight loss, reduced cholesterol levels, enhanced lipolysis and hepatic gluconeogenesis (3). Besides, recent studies have shown that altered thyroid status can affect circulating levels of several cytokines, including fibroblast growth factor 21 (FGF21), Fetuin A and Irisin (4, 5, 6, 7, 8), suggesting that these cytokines may be involved in the TH signaling network to modulate whole-body glucose and lipid homeostasis.

Neuregulin 4 (Nrg4), the fourth member of the neuregulin family, was originally identified in 1999 in adult pancreas (9). Nrg4 shares a common structure of epidermal growth factor (EGF)-like domains with other family members and activates type-1 growth factor receptors (ErbB3 and ErbB4 receptor) through tyrosine phosphorylation (9, 10). Subsequent studies showed that Nrg4 was expressed in some types of human malignancy and associated with high-grade tumors, suggesting its potential roles in cancer initiation and progression (11, 12, 13). Interestingly, recent studies demonstrated that Nrg4 is also highly expressed in metabolic organs or tissues, especially enriched in brown adipose tissues (BATs) and can be secreted as an adipokine (14, 15, 16). The BAT-derived Nrg4 activates ErbB3 and ErbB4 signaling in hepatocytes to inhibit hepatic lipogenesis and antagonize obesity-associated liver steatosis and insulin resistance (14, 15). Further studies showed that overexpression of Nrg4 reduces fat mass, increases energy expenditure and protects mice from high-fat-diet-induced obesity in part through activation of fatty acid oxidation and eliciting a healthy adipokine profile (17, 18). In contrast, mice depleted of Nrg4 or ErbB4 developed metabolic disorders as shown by development of obesity, dyslipidemia, hepatic steatosis, hyperglycemia, hyperinsulinemia and insulin resistance (14, 19).

Considering that both TH and Nrg4 have some overlap in metabolic effects, including the prevention of obesity and enhancing energy expenditure, we therefore tested the hypothesis that TH may increase circulating Nrg4 levels in humans and further characterize the source of Nrg4 in mice.

## Subjects and methods

### Human subjects

A total of 129 patients with hyperthyroidism were recruited from the Department of Endocrinology and Metabolism, Zhongshan Hospital (Shanghai, China) and Minhang Branch, Zhongshan Hospital (Shanghai, China).

Hyperthyroidism was diagnosed by typical clinical presentation, elevated serum TH, reduced thyroid-stimulating hormone (TSH) and elevated serum TSH receptor antibody (TRAb) levels. One hundred healthy controls were recruited from Department of Physical Examination Center, Zhongshan Hospital. Subjects with the following conditions were excluded: type 2 diabetes, cancer, pregnancy, lactation, subacute thyroiditis, abnormal liver function, abnormal renal function and infectious diseases. Thirty-nine hyperthyroid patients received thionamide treatment for 3 months and their TH levels reached the normal range. The study protocol was approved by the Human Research Ethical Committee of Zhongshan Hospital. All subjects provided written informed consents.

### Anthropometric and biochemical measurements

Body weight and height were obtained in light clothes and bare feet to the nearest 0.1 kg and 0.1 cm, respectively. Body mass index (BMI) was derived from weight in kilograms divided by square of height in meters. Fasting venous blood samples were collected from 08:00 to 09:00h after a 12-h overnight fast. Serum triglyceride (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), alanine transaminase (ALT), aspartate aminotransferase (AST), total bilirubin (TBIL) and direct bilirubin (DBIL) levels were measured with an auto-analyzer (Modular E170, Roche). Fasting blood glucose (FBG) was measured by glucose oxidase method on an auto-analyzer (Modular P800, Roche). Fasting insulin, free T<sub>3</sub>, free T<sub>4</sub> and TSH concentrations were measured by using electrochemiluminescence assays (Modular E170, Roche). The homeostasis model assessment of insulin resistance index (HOMA\_IR) was calculated as fasting insulin (μIU/ml) × fasting glucose (mmol/L)/22.5. Serum Nrg4 concentrations were measured using ELISA kits from Phoenix Pharmaceuticals Inc. (EK-056-24).

### Animal experiments

Male C57BL/6 mice aged 8–10 weeks were housed at 22 ± 1°C with a humidity of 35 ± 5% under a 12-h darkness/light cycle in a specific pathogen-free animal facility. Fifteen mice were divided into three groups and were injected intraperitoneally with either vehicle control (saline) or 3,3',5-triiodo-L-thyronine (T<sub>3</sub>, 0.5 mg/kg, #T2877, Sigma-Aldrich) for 4h (single injection) or 5 days (once daily). The primary hepatocytes were isolated as described previously (20) and maintained

in DMEM medium containing 10% fetal bovine serum. The fetal bovine serum was stripped with 10% resin to remove TH before use. Then cells were treated with either vehicle or 100 nM T<sub>3</sub> for 6 h. All protocols were approved by the Institutional Animal Care and Use Committee of Zhongshan Hospital, Fudan University (Shanghai, China).

### RNA extraction and quantitative real-time PCR

Total RNA was isolated from tissues or primary hepatocytes using TRIzol (Invitrogen) following the manufacturer's instructions. Quantitative real-time PCR was performed using a SYBR Green Premix Ex Taq (Takara) on Light Cycler480 (Roche). The primers were selected from PrimerBank (<https://pga.mgh.harvard.edu/primerbank/>): Nrg4 (Forward: 5'-ATGCCAACAGATC ACGAAGAG-3'; Reverse: 5'-AATGGGCTGGGAATAGTAGGT-3'); 36B4 (Forward: 5'-AGCCCAGAACACTGGTCTC-3'; Reverse: 5'-ACTCAGGATTCAA TGGTGCC-3'). The quantitative analysis of gene expression data was conducted using the 2<sup>-ΔΔCt</sup> method.

### Statistical analysis

All statistical analyses were performed using SAS 9.3 (SAS Institute). Normally distributed continuous variables were presented as means with standard deviations (s.d.), whereas skewed distributed continuous variables were presented as geometrical median and interquartile range.  $\chi^2$  and one-way ANOVA tests were used for comparison of categorical and continuous variables, respectively. The Student's paired *t* test was used for comparison of the data before and after anti-thyroid treatment. Skewed variables such as TSH and TPOAb were log-transformed before the analysis. Multivariate linear regression models were used to investigate the association of the following variables with Nrg4: BMI, free T<sub>3</sub>, free T<sub>4</sub>, TC, TG, HDL-C, ALT, AST, TBIL, DBIL, FBG, FINS. Multivariable logistic regression was performed to calculate the adjusted ORs and 95% CIs. A two-sided *P*<0.05 was considered statistically significant.

## Results

### Elevated serum Nrg4 levels in hyperthyroid subjects

The clinical characteristics of subjects in this study has been displayed in **Table 1**. The age and gender were comparable between hyperthyroid patients and normal subjects.

**Table 1** Clinical and biochemical features of hyperthyroid patients and control subjects.

Variables	Control	Hyperthyroid patients	P value
N	100	129	
Nrg4 (ng/ml)	2.21 ± 1.04	3.84 ± 1.63	<0.001
Free T <sub>3</sub> , pmol/L	5.4 ± 0.4	31.5 ± 12.1	<0.001
Free T <sub>4</sub> , pmol/L	16.4 ± 2.9	82.1 ± 36.4	<0.001
TSH, mIU/L	1.66 (0.98, 2.32)	0.01 (0.01, 0.01)	<0.001
TGAb, IU/mL	10.2 ± 3.1	34.2 ± 10.6	<0.001
TPOAb, IU/mL	4.20 (3.52, 4.66)	198.19 (116.51, 265.46)	<0.001
Age, years	33 ± 5	34 ± 5	0.166
Gender, male/female	65/35	85/44	0.890
BMI, kg/m <sup>2</sup>	22.7 ± 1.5	21.0 ± 2.1	<0.001
TC, mmol/L	4.61 ± 0.53	3.46 ± 0.77	<0.001
TG, mmol/L	1.13 ± 0.31	1.17 ± 0.34	0.363
HDL-C, mmol/L	1.24 ± 0.34	1.18 ± 0.45	0.209
ALT, U/L	23.6 ± 5.3	35.0 ± 9.8	<0.001
AST, U/L	26.7 ± 10.0	41.5 ± 11.5	<0.001
TBIL, μmol/L	13.2 ± 5.9	13.9 ± 5.1	0.325
DBIL, μmol/L	2.90 ± 1.05	2.65 ± 1.29	0.117
FBG, mmol/L	5.18 ± 0.49	5.30 ± 0.59	0.116
FINS, mU/L	10.7 ± 4.1	12.4 ± 4.6	0.003
HOMA_IR	2.44 ± 0.94	2.93 ± 1.08	0.001
Heart rate, bpm	77 ± 11	109 ± 13	<0.001

Data are expressed as mean ± s.d.

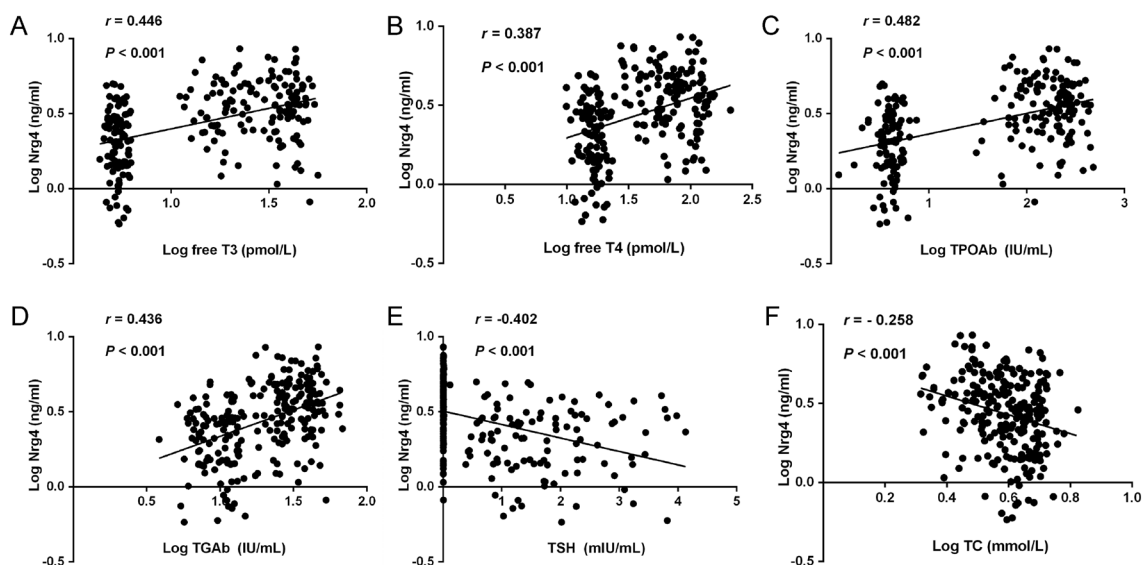
ALT, alanine transaminase; AST, aspartate aminotransferase; BMI, body mass index; DBIL, direct bilirubin; FBG, fasting blood glucose; FINS, fasting insulin; HDL-C, high-density lipoprotein cholesterol; HOMA\_IR, the homeostasis model assessment of insulin resistance index; Nrg4, neuregulin 4; TBIL, total bilirubin; TC, total cholesterol; TG, triglyceride; TGAb, thyroglobulin antibody; TPOAb, anti-thyroid peroxidase antibody; TSH, thyroid-stimulating hormone.

Patients with hyperthyroidism exhibited increased levels of free T<sub>3</sub>, free T<sub>4</sub>, ALT, AST, FINS, HOMA\_IR and heart rate, decreased levels of BMI, TC and TSH. Besides, serum TG, HDL-C, TBIL, DBIL and FBG levels were generally the same between the case-control groups. As shown in **Table 1**, serum Nrg4 concentrations were dramatically increased in hyperthyroid patients compared with normal subjects (3.84 ± 1.63 vs 2.21 ± 1.04, *P*<0.001).

### Association of serum Nrg4 levels with hyperthyroidism

In all the subjects, serum Nrg4 levels were positively correlated with free T<sub>3</sub>, free T<sub>4</sub>, TPOAb and TGAb levels (**Fig. 1A, B, C and D**, *P*<0.001). Moreover, serum Nrg4 levels were negatively correlated with TSH and TC levels (**Fig. 1E and F**, *P*<0.001). In the multivariate linear regression model, FT<sub>3</sub> and AST were positively associated with Nrg4 (**Table 2**).

Further logistic regression models were performed to explore the unadjusted and adjusted ORs with associated 95% CI of serum Nrg4 for hyperthyroidism (**Table 3**).



**Figure 1** Correlation of Nrg4 with serum levels of free T4 (A), free T3 (B), TPOAb (C), TGAb (D), TSH (E) and total cholesterol (TC) (F).

In model 1, with no adjustment for any confounding factor, per SD increase of serum Nrg4 levels was significantly associated with hyperthyroidism (OR, 4.89; 95% CI, 3.16–8.02;  $P < 0.001$ ). In model 2, after adjustment for age, gender and BMI, per SD increase of serum Nrg4 levels was also significantly associated with hyperthyroidism (OR, 5.62; 95% CI, 3.41–10.01;  $P < 0.001$ ). In model 3, after further adjustment for ALT, AST, FBG, FINS and TC, per SD increase of serum Nrg4 levels remained independently associated with hyperthyroidism, with the adjusted OR (95% CI) of 5.86 (2.32–18.37) ( $P < 0.001$ ).

### Reduced serum Nrg4 levels in patients after thionamide treatment

Furthermore, 39 hyperthyroidism patients received thionamide treatment and euthyroid levels were achieved after 3 months. Accordingly, free  $T_3$ , free  $T_4$ , ALT, AST and heart rate were decreased, whereas body weight, TC and TSH were increased. In parallel, serum Nrg4 concentration were dramatically decreased in patients after thionamide treatment ( $3.57 \pm 1.26$  vs  $1.94 \pm 0.72$ ,  $P < 0.001$ ) (Table 4).

### TH treatment upregulates Nrg4 expression in mice

To determine which organ or tissue may contribute to the elevated circulating Nrg4 in hyperthyroid patients, C57BL/6 mice were treated with  $T_3$  intraperitoneally for 4h to evaluate the acute effect or once daily for 5 days to evaluate chronic effect, respectively. As shown in

Fig. 2A and B, mRNA levels of Nrg4 were moderately but significantly induced in the inguinal and epididymal white adipose tissues (WATs) after administration of  $T_3$ . Notably, its mRNA expression was also dramatically induced in the livers of mice treated with  $T_3$  (Fig. 2C). In agreement,  $T_3$  treatment increased Nrg4 mRNA levels in mouse primary hepatocytes (Fig. 2D). In contrast, its expression in the BATs and skeletal muscles (SKMs) were not altered (Fig. 2E and F). Therefore, our results suggest that the WAT and liver appear to be the primary source for circulating Nrg4 levels by  $T_3$  treatment. Serum Nrg4 levels were not determined because a reliable immunoassay for mouse circulating Nrg4 was not available at present (14).

**Table 2** Multiple linear regression analysis of variables associated with serum Nrg4.

	$\beta \pm$ s.e.	P value
BMI, kg/m <sup>2</sup>	-0.02 ± 0.05	0.66
Free $T_3$ , pmol/L	0.04 ± 0.01	<0.01
Free $T_4$ , pmol/L	-0.002 ± 0.003	0.52
TC, mmol/L	-0.07 ± 0.13	0.59
TG, mmol/L	-0.30 ± 0.30	0.31
HDL-C, mmol/L	0.45 ± 0.24	0.06
ALT, U/L	0.005 ± 0.01	0.69
AST, U/L	0.03 ± 0.01	0.01
TBIL, $\mu$ mol/L	0.005 ± 0.02	0.77
DBIL, $\mu$ mol/L	-0.05 ± 0.08	0.52
FBG, mmol/L	-0.20 ± 0.18	0.25
FINS, mU/L	-0.02 ± 0.02	0.32

$\beta$ , regression coefficient.

**Table 3** ORs for association of serum Nrg4 levels with hyperthyroidism.

Variable	OR (95% CI)	P value
Model 1		
Nrg4 <sup>a</sup>	4.89 (3.16–8.02)	<0.01
Model 2		
Nrg4 <sup>a</sup>	5.62 (3.41–10.01)	<0.01
Age	1.03 (0.97–1.11)	0.34
Gender (female vs male)	0.69 (0.32–1.47)	0.34
BMI	0.56 (0.46–0.70)	<0.01
Model 3		
Nrg4 <sup>a</sup>	5.86 (2.32–18.37)	<0.01
Age	1.00 (0.88–1.13)	0.96
Gender (female vs male)	0.37 (0.09–1.47)	0.16
BMI	0.52 (0.35–0.78)	<0.01
ALT	1.27 (1.13–1.43)	<0.01
AST	1.05 (0.98–1.13)	0.14
FBG	3.22 (0.72–14.34)	0.13
FINS	1.12 (0.96–1.32)	0.15
TC	0.03 (0.01–0.14)	<0.01

<sup>a</sup>OR and 95% CI expressed as per s.d. increase of Nrg4.

## Discussion

In the present study, our results suggest that TH has a strong impact on the circulating Nrg4 levels. First, Nrg4 levels were significantly higher in patients with hyperthyroidism compared with euthyroid controls and dramatically decreased after thionamide treatment.

**Table 4** Serum NRG4 levels before and after thionamide treatment in hyperthyroid patients.

	Before	After	P value
<i>n</i>	39	39	
Nrg4 (ng/ml)	3.57 ± 1.26	1.94 ± 0.72	<0.001
Free T <sub>3</sub> , pmol/L	27.1 ± 20.0	4.6 ± 0.8	<0.001
Free T <sub>4</sub> , pmol/L	61.3 ± 26.6	13.1 ± 2.7	<0.001
TSH, mIU/L	0.01 (0.01, 0.01)	0.45 (0.01, 1.16)	<0.001
Weight, kg	52.0 ± 6.2	56.8 ± 7.3	<0.001
ALT, U/L	37.7 ± 15.9	20.6 ± 9.6	<0.001
AST, U/L	31.1 ± 11.9	20.6 ± 7.1	<0.001
TBIL, μmol/L	14.9 ± 7.5	12.3 ± 4.3	0.056
DBIL, μmol/L	4.75 ± 3.22	3.71 ± 1.56	0.069
TC, mmol/L	3.71 ± 0.81	5.44 ± 0.99	<0.001
TG, mmol/L	0.99 ± 0.72	1.08 ± 0.63	0.476
HDL-C, mmol/L	1.40 ± 0.39	1.66 ± 0.33	0.002
FBG, mmol/L	5.20 ± 0.70	5.02 ± 0.41	0.163
FINS, mIU/L	11.2 ± 13.7	8.9 ± 7.1	0.340
HOMA <sub>1R</sub>	2.67 ± 3.22	2.01 ± 1.63	0.244
Heart rate, bpm	112 ± 12	79 ± 7	<0.001

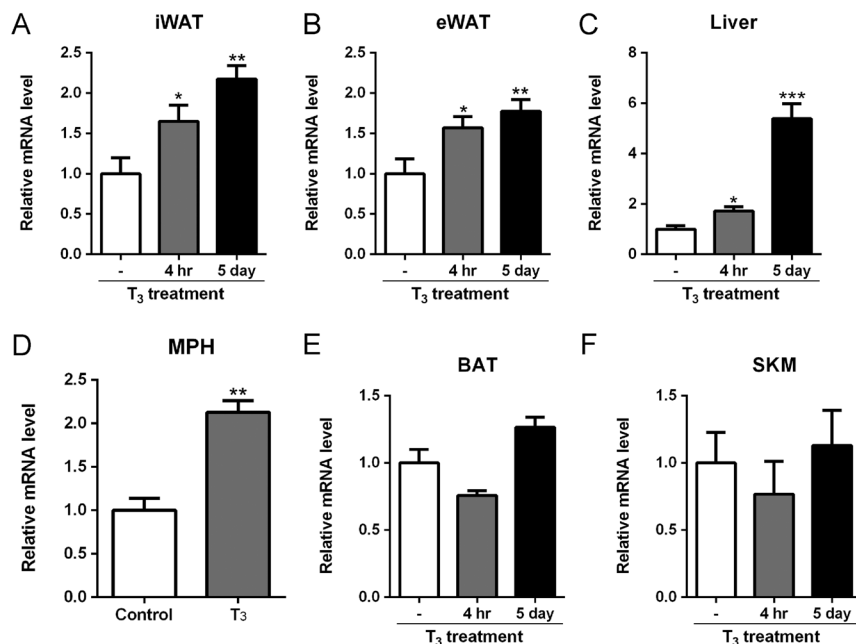
Data are expressed as mean ± s.d.

Second, both correlation and logistic regression analysis confirmed the association of serum Nrg4 levels with thyroid status. Third, we showed that Nrg4 expression levels were significantly upregulated in WAT and livers in mice by both acute and chronic T<sub>3</sub> treatment. Finally, we provided *ex vivo* evidence showing that Nrg4 expression in primary hepatocytes were also induced by T<sub>3</sub> treatment. Therefore, our results provide the clinical, animal and cellular results showing that Nrg4 was associated with hyperthyroidism.

Initial studies demonstrated that Nrg4 is expressed in the adult pancreas and is essential for delineation of the somatostatin-producing delta-cells in developing pancreatic islets (9). Nrg4 further obtained great attention since Wang *et al.* reported that Nrg4 is enriched in brown and WAT and preserves metabolic homeostasis through attenuation of hepatic lipogenesis (14). A subsequent study also proposed that hepatic Nrg4 signaling could be an endocrine checkpoint for steatosis-to-steatohepatitis progression by countering stress-induced liver injury, fibrosis and cell death (21). In addition, several studies in human cohorts revealed that Nrg4 levels were increased in patients with prediabetes, newly diagnosed diabetes, gestational diabetes or polycystic ovary syndrome, suggesting Nrg4 concentrations were associated with the development and progression of insulin resistance (22, 23, 24, 25). However, other findings indicate that circulating Nrg4 concentrations may be a protective factor in the development of metabolic syndrome. For instance, in newly diagnosed patients with type 2 diabetes, lower plasma Nrg4 levels were associated with elevated high-sensitivity C-reactive protein (26). In obese adults, subjects with low levels of circulating Nrg4 had high prevalence of fasting glucose and blood pressure (27). Interestingly, our data found that circulating Nrg4 levels were negatively correlated with serum TC levels. Consistently, Jiang *et al.* reported that lower circulating Nrg4 concentrations are associated with subclinical atherosclerosis in obese adults, as shown by increased carotid intima-media thickness and atherosclerotic plaque (28). Therefore, circulating Nrg4 might play a protective role in cardiovascular diseases.

Our animal studies further indicate that the liver and WAT appeared to be major sources of Nrg4 in prolonged hyperthyroidism. Wang *et al.* reported that Nrg4 mRNA was enriched in adipose tissue and relatively lower in other tissues (14). However, Liu *et al.* reported that hepatic Nrg4 expression was significantly upregulated in the liver in response to experimental myocardial ischemia (29), suggesting that it may contribute to cardioprotection and could be considered for the development of



**Figure 2**

TH induces Nrg4 expression in C57BL/6 mice and primary hepatocytes. (A, B and C) Relative mRNA levels of Nrg4 in inguinal white adipose tissue (iWAT, A), epididymal white adipose tissue (eWAT, B) and liver (C) tissues of mice treated with T<sub>3</sub> or vehicle control for 4 h or 5 days ( $n = 5$  per group). (D) Relative mRNA levels of Nrg4 in mouse primary hepatocytes (MPH) treated with T<sub>3</sub> for 6 h. (E and F) Relative mRNA levels of Nrg4 in brown adipose tissue (BAT, E) and skeletal muscle (SKM, F) of mice treated with T<sub>3</sub> or vehicle control for 4 h or 5 days ( $n = 5$  per group). Data are presented as means  $\pm$  standard error. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ .

cardioprotective agents. Therefore, together with our findings, these studies indicate that the expression of Nrg4 in the liver might be also important under certain physiological or pathological conditions.

TH is required for regulating metabolism in the adult, through its two receptor isoforms,  $\alpha$  and  $\beta$ , which are differentially expressed in tissues and have distinct functions in mediating TH signaling. It has been well established that TH status correlates with body weight and energy expenditure (30). In adipocytes, TH can directly stimulate thermogenesis in BATs and also induce WAT browning through peripheral and central mechanisms (31). In the liver, TH regulates cholesterol and bile acid homeostasis through direct actions on gene expression as well as cross-talk with other nuclear receptors (30). TH also stimulates gluconeogenesis and hepatic glucose production through the regulation of phosphoenolpyruvate carboxykinase (PEPCK) (32, 33). In addition, recent studies have demonstrated that impaired thyroid action is associated with nonalcoholic fatty liver disease (NAFLD), and selective TH receptor agonists have exhibited strong effects against NAFLD in preliminary clinical trials (34, 35, 36). Taken together with the results of the current study, Nrg4 plays an important role in preserving metabolic homeostasis and have some overlap in metabolic effects with TH, including attenuating hepatic lipogenesis (14), increasing energy expenditure (18, 37) and maintaining hepatic gluconeogenesis (38), it is tempting to assume that partial metabolic benefits of TH might be mediated by the upregulation of Nrg4 expression.

Further studies using Nrg4 knockout and transgenic mice will clarify potential metabolic mechanisms connecting Nrg4 and thyroid function/hormones.

There are several limitations of this study that we would like to point out: First, this study was cross-sectional and the sample size for hypothyroid patients was relatively small. Second, the reason for the tissue-specific effects of TH in the regulation of Nrg4 expression remains explored. We analyzed the promoter (from position  $-2500$ bp to transcription start site) and introns of *Nrg4* gene and did not identify a classic TR response element in those regions. Therefore, whether TH activates Nrg4 mRNA transcription through a direct or indirect role needs further investigations.

In conclusion, we, for the first time to our knowledge, showed that serum Nrg4 levels are independently associated with hyperthyroidism. TH might be an important stimulus that increases endogenous Nrg4 expression.

#### Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

#### Funding

This study is supported by grants from Science and Technology Commission of Shanghai Municipality (Nos. 18dz2304400 and 15411970700), Natural Science Foundation of China (Nos. 81471016 and 81500603) and the Pujiang Talent Program from Science and Technology Commission of Shanghai Municipality.

### Author contribution statement

Y S, J Y and L Z conceived the project. M L, J J, Y L, S Z, C S and H Y performed the animal and cellular experiments. Y C, Z S and X F took statistical analysis. M L, Y C, J J, Y L and L Z drafted the manuscript. All authors reviewed the manuscript.

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Received in final form 22 April 2019

Accepted 7 May 2019

Accepted Preprint published online 7 May 2019