

CLINICAL STUDY

Serum vaspin levels in hypothyroid patients

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Abstract

Objective: To elucidate the link between TSH and obesity, the relationship between TSH and adipocytokines were previously studied. Animal studies demonstrated a possible relationship between vaspin levels and thyroid functions. In this study, we aimed to investigate vaspin levels in hypothyroid states and its relationship with insulin resistance parameters in humans.

Design: Prospective observational study.

Methods: We enrolled 27 overt hypothyroid, 33 subclinical hypothyroid and 41 euthyroid patients. We measured the body mass index (BMI), fasting glucose, fasting insulin, homeostasis model assessment of insulin resistance index (HOMA-IR), lipid profile, TSH, free triiodothyronine, free thyroxine and vaspin levels. The change in vaspin levels in 12 overt hypothyroid patients after establishment of euthyroidism was analysed.

Results: All groups were age-matched. Overt hypothyroid group had higher BMI values ($P < 0.05$) than other groups. No significant difference was observed in insulin levels and HOMA-IR among the groups ($P > 0.05$). Adjusted vaspin levels for BMI and age were similar among the groups. Mean vaspin levels in overt, subclinical and euthyroid patients were 1.20 ± 1.17 , 1.48 ± 0.93 and 0.95 ± 0.75 ng/ml respectively ($P > 0.05$). There was no significant association between vaspin levels and BMI, fasting glucose, insulin and HOMA-IR ($P > 0.05$). Establishing euthyroidism in hypothyroid patients did not result in a significant change in vaspin levels (before and after treatment, 1.35 ± 1.06 and 1.25 ± 0.68 ng/ml, respectively; $P > 0.05$).

Conclusion: We herein present novel data indicating vaspin levels are neither altered in overt and subclinical hypothyroidism nor have a relationship with features of insulin resistance in hypothyroid patients.

European Journal of Endocrinology 165 563–569

Introduction

Adipose tissue acts as an endocrine organ and plays a great role in the pathogenesis of insulin resistance, diabetes and atherosclerosis by means of adipocyte-derived factors (adipocytokines). It is known that visceral adipose tissue (VAT) carries a greater metabolic risk than s.c. adipose tissue. Recently, Hida *et al.* (1) identified a novel insulin-sensitising adipocytokine called VAT-derived serine protease inhibitor (vaspin) from the VAT of obese, diabetic Otsuka Long-Evans Tokushima Fatty (OLETF) rats. Vaspin was found to be a member of the serine protease inhibitor family (serpins). They reported that administration of vaspin to OLETF rats caused improvement in glucose tolerance and insulin sensitivity (1). Also, recent studies have documented positive association between vaspin gene expression in human adipose tissue and serum vaspin levels with obesity and type 2 diabetes (2–4).

Hypothyroidism has been associated with hypertension, dyslipidaemia, metabolic syndrome and increased cardiovascular risk. Insulin resistance plays major role

in the development of metabolic syndrome. The data about the relationship between insulin resistance and hypothyroidism is still controversial. Some authors suggest that elevated TSH as a metabolic adaptation to obesity while others suggest that elevated TSH is associated with insulin resistance, dyslipidaemia and subclinical inflammation resulting in an increased risk of coronary artery disease. Even slightly higher TSH within the normal reference range has been linked to higher body mass index (BMI), dyslipidaemia and fatal coronary artery disease. Decreased insulin sensitivity was reported in some studies (5, 6) while others could not find any association (7, 8). TSH is often elevated in obesity associated with disorders of lipid and glucose metabolism.

To elucidate the link between TSH and obesity, the relationship between TSH and adipocytokines were studied. Various adipocytokines such as leptin, adiponectin, interleukin 6, tumor necrosis factor α , visfatin and resistin have already been studied in hypothyroidism but controversial results were reported (9–11). The most promising adipocytokine is leptin.

Leptin and TSH have similar circadian rhythms and leptin deficiency is associated with dysregulation in circadian rhythms of TSH. Leptin stimulates TSH production via hypothalamic–pituitary axis and TSH stimulates leptin production in adipocytes (12, 13). Weight loss was associated with a modulation of thyroid function that might be mediated by changes in leptin levels. The relationship of vaspin levels with hypothyroidism in humans was not studied to date. Two studies indicating a possible relationship between vaspin levels and thyroid dysfunction were conducted. One of them is an animal study that demonstrated that vaspin mRNA levels were significantly downregulated in hyperthyroid rats and increased in hypothyroid rats with respect to euthyroid rats (14). The other study showed a significant reduction in TSH levels after Roux-En-Y Gastric Bypass (RYGB), which was positively correlated with leptin and vaspin serum concentrations (15).

In this study, we aimed to investigate vaspin levels and its relationship with both anthropometric and insulin resistance parameters in subclinical and overt hypothyroid patients.

Methods

Subjects

In this study, 60 hypothyroid patients and 41 controls were enrolled. Twenty-seven of the patients were diagnosed as overt hypothyroid and 33 of them were diagnosed as subclinical hypothyroid. The study protocol was approved by the Ethics Committee of Hacettepe University School of Medicine and all participants gave their written informed consent before participating the study. Subjects with diabetes mellitus, renal or hepatic disease, acute or chronic inflammatory disease, cancer, acute or chronic infections, using any medication affecting thyroid function tests were excluded from the study. The control subjects were not suffering from any health problems and were not receiving any medications. We categorised the patients according to TSH ranges as follows: 0.36–4.5 (euthyroid group), 4.6–10 (subclinical hypothyroid group) and > 10 µU/ml (overt hypothyroid group) (16). We performed two determinations of TSH to confirm the diagnosis of subclinical and overt hypothyroidism. We measured anti-thyroid peroxidase and anti-thyroglobulin levels in all subclinical and overt hypothyroid patients and thyroid ultrasonography was performed in all patients. The patients with autoimmune thyroid disease (confirmation with antibody positivity or sonographic appearance of thyroiditis) were included in the study. Overt hypothyroid patients were given Levothyroxine (LT₄) sodium at a dose of 1.7 µg/kg per day. In patients with cardiovascular disease, LT₄ sodium was started in small doses and slowly titrated. In a small group of patients (*n* = 12), TSH levels were re-evaluated 2 months

after the beginning of treatment and dose was titrated until the establishment of euthyroidism. These patients became euthyroid in an average of 3.8 ± 1.4 months (range 2–6 months).

All subjects underwent a physical examination, detailed history and anthropometric measurements including BMI, waist-to-hip ratio (WHR) and blood pressure. BMI was calculated as weight divided by squared height (in kilograms per square metre). Waist circumference was measured from the narrowest point between the lowest rib and the uppermost lateral border of the iliac crest. The hip circumference was measured at their widest point. Blood pressure was measured twice in the same arm of each patient at rest for a minimum of 15 min and the mean value of the two measurements was used.

Laboratory analyses

Blood samples were drawn after an overnight fasting. They were centrifuged and immediately frozen in aliquots and stored at -80°C until analysis. Triglycerides (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C) were measured using enzymatic calorimetric kits with intra- and inter-assay coefficients of variation (CV) of <10% (Roche Diagnostics GmbH). Fasting plasma glucose (FPG) concentration was determined by the glucose oxidase method (Olympus AU 2700, Beckman Coulter Inc.). Thyroid hormone levels, including TSH, free T₄ (FT₄) and free triiodothyronine (FT₃), were measured by electrochemiluminescence immunoassay method (Roche Diagnostics GmbH). Serum fasting insulin was determined by chemiluminescence method (Roche Diagnostics GmbH). The average intra- and inter-assay CV for insulin were ≤ 4.3 and $\leq 3.4\%$ respectively. Insulin resistance was quantified by the homeostasis model assessment of insulin resistance index (HOMA-IR) calculated from the following formula: $\text{HOMA-IR} = \text{fasting insulin (in microunits per millilitre)} \times \text{fasting glucose (in milligrams per deciliter)} / 405$ (17). Serum vaspin levels were determined by a commercially available ELISA according to the manufacturer's instructions (Adipogen, Seoul, South Korea). The average intra- and inter-assay CV for vaspin were ≤ 3.6 and $\leq 9.06\%$ respectively. Serum thyroid hormone levels (TSH, FT₃ and FT₄), lipid profile, fasting insulin, glucose and vaspin levels were measured after establishing euthyroidism in overt hypothyroid patients.

Statistical analysis

Statistical analysis was performed by the Statistical Package for the Social Sciences (SPSS v.11.0, Chicago IL, USA). Results were presented as mean \pm s.d. Normality of distribution was evaluated with Kolmogorov–Smirnov test. Statistical differences among the groups were analysed by one-way ANOVA followed by the Tukey's

post hoc test for parametric variables. Non-parametric variables were analysed by Kruskal–Wallis and Mann–Whitney *U* test. Correlations among the study variables were tested by the Pearson or Spearman correlation coefficient. Analysis of covariance (ANCOVA) was used to adjust variables for BMI and age. A value of $P < 0.05$ was considered as statistically significant.

Results

The baseline general characteristics of the study population are given in Table 1. In total 82 female (81.2%) and 19 male (18.8%) patients were included in the study. There was no significant difference in the distribution of gender between the groups ($P > 0.05$). Overt hypothyroid patients had higher BMI than subclinical hypothyroid and euthyroid group (Table 1, $P < 0.05$). Age, FPG, insulin and HOMA-IR were similar among the groups (Table 1). Patients with overt hypothyroidism had higher TG and lower HDL levels than euthyroid controls (Table 1, $P < 0.05$), which disappeared after adjustment for BMI ($P > 0.05$).

There was no difference in vaspin levels among the three groups (Table 1, $P > 0.05$). After adjustment for BMI and age, a non-significant difference in vaspin levels between subclinical hypothyroid and euthyroid group was observed at a level of $P = 0.063$. Also, there was no significant correlation between vaspin levels and BMI, WHR, FPG, fasting insulin and HOMA-IR values ($P > 0.05$). Vaspin levels were not correlated with TSH, FT₃ and FT₄ levels ($r = 0.082$, -0.070 and -0.083 , respectively; $P > 0.05$ for all). In each group, the

analysis of vaspin in relation to BMI was calculated, but no significant correlation was observed ($P > 0.05$).

Owing to gender difference in vaspin levels (3, 18) and small number of males included in our study, we further evaluated the relationship between vaspin levels and thyroid status in women. The baseline general characteristics of the women in our study group are given in Table 2. After adjustment for BMI, only FPG levels remained significant ($P < 0.05$) between the groups. Similar to the analysis including whole patients, the analysis in women revealed no significant difference in vaspin levels among the groups. Even after adjustment for age and BMI, vaspin levels were similar between the groups (Table 2, $P = 0.077$). We failed to find any correlation between vaspin levels and TSH, BMI, FPG, fasting insulin and HOMA-IR values in women ($P > 0.05$).

When we analysed the correlations between TSH levels and lipid profile, FPG, fasting insulin and HOMA-IR levels in the entire study cohort, we found a significant correlation between TSH and TC, TG and LDL-C levels ($r = 0.323$, 0.231 and 0.327 , respectively; $P < 0.05$ for all). After controlling for BMI, TSH was still correlated with TC and LDL-C levels but not with TG ($r = 0.287$, $P < 0.05$; $r = 0.280$, $P < 0.05$; $r = 0.158$, $P > 0.05$ respectively). TSH, FT₄ and FT₃ were not correlated with FPG, insulin and HOMA-IR values ($P > 0.05$).

We further analysed serum vaspin levels of 12 overt hypothyroid patients after normalisation of thyroid function. Treatment with LT₄ sodium did not alter vaspin levels in overt hypothyroid patients (Table 3, $P > 0.05$).

Table 1 Clinical and laboratory characteristics of the study groups. Results are expressed as mean \pm s.d. (range).

	Overt hypothyroid	Subclinical hypothyroid	Euthyroid control	<i>P</i>	<i>P</i> ₁	<i>P</i> ₂	<i>P</i> ₃
<i>n</i>	27	33	41		–	–	–
Age (years)	40.74 \pm 11.80 (16–58)	39.0 \pm 13.58 (17–62)	35.37 \pm 10.52 (18–63)	NS			
Sex (M/F)	2 M/25 F	7 M/26 F	10 M/31 F	NS			
BMI (kg/m ²)	30.04 \pm 6.35 (17.63–41.26)	25.82 \pm 5.59 (16.65–35.63)	25.91 \pm 5.30 (17.24–37.66)	<0.05	<0.05	<0.05	NS
WHR	0.84 \pm 0.11 (0.76–1.02)	0.84 \pm 0.15 (0.67–1.53)	0.82 \pm 0.09 (0.67–0.98)	NS			
SBP (mmHg)	122.52 \pm 21.00 (86–170)	120.0 \pm 18.03 (85–159)	118.31 \pm 14.51 (99–160)	NS			
DBP (mmHg)	80.16 \pm 10.90 (60–100)	75.64 \pm 10.17 (50–92)	77.17 \pm 11.64 (53–108)	NS			
TSH (μ U/ml)	37.65 \pm 31.77 (12.2–100)	6.75 \pm 1.67 (4.6–9.89)	2.33 \pm 1.16 (0.39–4.59)	<0.001	<0.001	<0.001	<0.001
FT ₃ (pmol/l)	4.39 \pm 1.42 (1.70–8.89)	4.90 \pm 0.71 (3.74–6.78)	4.86 \pm 0.57 (4.07–6.26)	NS			
FT ₄ (pmol/l)	9.07 \pm 3.01 (3.51–14.08)	14.24 \pm 2.14 (10.24–18.2)	15.61 \pm 1.80 (11.56–19.30)	<0.001	<0.001	<0.001	<0.05
FPG (mg/dl)	91.15 \pm 13.43 (67–120)	92.24 \pm 10.79 (67–119)	86.74 \pm 12.24 (62–113)	NS			
Insulin (μ U/ml)	12.43 \pm 5.93 (5.7–24)	9.87 \pm 4.88 (2.1–22.0)	10.27 \pm 4.09 (2.9–20.0)	NS			
HOMA-IR	2.89 \pm 1.69 (1.29–6.76)	2.28 \pm 1.20 (0.35–5.81)	2.21 \pm 0.96 (0.51–4.49)	NS			
TC (mg/dl)	201.67 \pm 51.12 (113–306)	183.15 \pm 41.80 (119–273)	181.00 \pm 40.95 (87–288)	NS			
TG (mg/dl)	140.77 \pm 74.83 (41–363)	102.97 \pm 43.27 (46–248)	98.78 \pm 75.38 (34–470)	<0.05	NS	<0.05	NS
HDL-C (mg/dl)	48.96 \pm 12.30 (30.2–72.7)	53.89 \pm 15.44 (33–99)	58.15 \pm 14.72 (29–92)	<0.05	NS	<0.05	NS
LDL-C (mg/dl)	126.44 \pm 45.27 (60–216)	113.97 \pm 33.94 (64–185)	108.12 \pm 34.37 (44–200)	NS			
Vaspin (ng/ml)	1.23 \pm 0.89 (0.23–4.22)	1.42 \pm 1.13 (0.19–5.81)	0.99 \pm 0.74 (0.21–3.38)	NS			
Vaspin adj (ng/ml) ^a	1.20 \pm 1.17	1.48 \pm 0.93	0.95 \pm 0.75	NS	NS	NS	0.063

P for three groups: *P*₁, for overt hypothyroid and subclinical hypothyroid group; *P*₂, for overt hypothyroid and euthyroid control group; *P*₃, for subclinical hypothyroid and euthyroid control group; NS, Non-significant.

^aMean \pm s.d. by general linear model with adjustment of age and BMI.

Table 2 Clinical and laboratory characteristics of the women in the study group. Results are expressed as mean \pm s.d.

	Overt hypothyroid	Subclinical hypothyroid	Euthyroid control	P	P1	P2	P3
n	25	26	31		–	–	–
Age (years)	40.68 \pm 12.18	39.27 \pm 12.91	34.35 \pm 10.81	NS			
BMI (kg/m ²)	30.12 \pm 6.62	26.29 \pm 5.76	25.22 \pm 5.25	<0.05	NS	<0.05	NS
WHR	0.88 \pm 0.06	0.84 \pm 0.17	0.79 \pm 0.07	<0.05	NS	<0.05	NS
SBP (mmHg)	120.96 \pm 21.04	119.42 \pm 19.15	116.32 \pm 14.98	NS			
DBP (mmHg)	79.65 \pm 11.08	74.88 \pm 10.55	76.12 \pm 12.51	NS			
TSH (μ U/ml)	39.07 \pm 32.62	6.82 \pm 1.69	2.48 \pm 1.21	<0.001	<0.001	<0.001	<0.001
FT ₃ (pmol/l)	4.32 \pm 1.45	4.86 \pm 0.75	4.76 \pm 0.52	NS			
FT ₄ (pmol/l)	8.88 \pm 3.04	14.23 \pm 2.17	15.44 \pm 1.83	<0.001	<0.001	<0.001	NS
FPG (mg/dl)	90.12 \pm 13.35	93.81 \pm 11.52	84.5 \pm 11.42	<0.05	NS	NS	<0.05
Insulin (μ U/ml)	11.62 \pm 5.59	10.27 \pm 4.48	10.40 \pm 4.21	NS			
HOMA-IR	2.66 \pm 1.59	2.41 \pm 1.17	2.17 \pm 0.95	NS			
TC (mg/dl)	200.96 \pm 52.51	185.42 \pm 45.41	171.87 \pm 37.45	NS			
TG (mg/dl)	141.28 \pm 76.33	102.77 \pm 47.83	85.77 \pm 45.79	<0.05	NS	<0.05	NS
HDL-C (mg/dl)	49.87 \pm 11.05	54.27 \pm 17.00	58.92 \pm 14.26	NS			
LDL-C (mg/dl)	128.52 \pm 45.92	117.38 \pm 35.93	100.97 \pm 30.78	<0.05	NS	<0.05	NS
Vaspin (ng/ml)	1.17 \pm 0.87	1.64 \pm 1.17	1.11 \pm 0.79	NS			
Vaspin adj (ng/ml) ^a	1.14 \pm 1.09	1.63 \pm 0.96	1.07 \pm 0.88	NS			

P for three groups: P1, for overt hypothyroid and subclinical hypothyroid group; P2, for overt hypothyroid and euthyroid control group; P3, for subclinical hypothyroid and euthyroid control group; NS, Non-significant.

^aMean \pm s.d. by general linear model with adjustment of age and BMI.

Discussion

The relationship between hypothyroidism, obesity and insulin resistance is of clinical interest (19–22). To elucidate the link between thyroid disorders and adipose tissue, various adipocytokines were previously studied (9–11), in which the most promising one was leptin. Controversial results are present about the relationship between thyroid disease and adipocytokines studied to date. Vaspin is a novel adipocytokine, i.e. supposed to have insulin-sensitising effects. Vaspin mRNA was found to be altered in hypothyroid and hyperthyroid rats (14). We herein present novel data that vaspin levels are neither altered in overt and subclinical hypothyroidism nor have a relationship with features of insulin resistance in hypothyroid patients.

Metabolic syndrome is known as a cluster of obesity, hyperglycaemia, dyslipidaemia and hypertension. The association between serum TSH and the components of metabolic syndrome varied in different studies. In some recent studies, thyroid dysfunction (23) and even TSH within the normal range (19, 24, 25) were reported to be associated with insulin resistance and components of metabolic syndrome. By contrast, some other studies failed to detect a significant association between TSH and insulin resistance (19, 21, 22, 26). It is difficult to prove a casual relationship between insulin resistance and thyroid hormones as both are influenced by weight status. Bastemir *et al.* (27) found that the relationship between thyroid hormones and features of insulin resistance disappeared after adjustment for BMI. Similar to the studies that failed to find an association between thyroid dysfunction and insulin resistance, hypothyroidism did not influence fasting insulin levels

and HOMA-IR values in our study. Only overt hypothyroid patients had non-significantly higher values than other groups.

Vaspin is a novel adipocytokine with potential insulin-sensitising effects. In humans, expression of vaspin (SERPINA12) mRNA was detected in human VAT and subcutaneous adipose tissue (2). Vaspin expression was shown to increase from overweight to obesity and detected in patients with type 2 diabetes mellitus rather than individuals with normal glucose tolerance (2). In obese children, serum vaspin was positively correlated with TG, fasting insulin and HOMA-IR (28). In overweight women with polycystic ovarian syndrome, serum vaspin levels and its expression in omental adipose tissue were significantly elevated and decreased by metformin treatment in accordance with the decrease in insulin resistance (29). They also detected a significant positive association with vaspin and BMI and WHR. In a study including 150 subjects, a positive correlation between serum vaspin concentrations and the VAT area was shown (30). El-Mesallamy *et al.* (4)

Table 3 Change in the levels of vaspin after thyroid hormone replacement in overt hypothyroid patients. Results are expressed as mean \pm s.d.

	Before treatment (n=12)	After treatment (n=12)	P
Vaspin (ng/ml)	1.35 \pm 1.06	1.25 \pm 0.68	NS
TSH (μ U/ml)	32.33 \pm 23.68	1.86 \pm 1.64	<0.05
FT ₃ (pmol/l)	4.11 \pm 1.29	5.16 \pm 1.29	<0.05
FT ₄ (pmol/l)	8.89 \pm 2.56	19.16 \pm 4.55	<0.001

NS, Non-significant.

reported higher levels of vaspin in both non-obese and obese type 2 diabetes patients than control subjects. In contrast, serum vaspin levels were reported to be associated with BMI and insulin sensitivity in subjects with normal glucose tolerance but not in patients with type 2 diabetes mellitus (3). We previously showed comparable vaspin levels in type 2 diabetic patients and controls but lower vaspin levels in the ones with microvascular complications (31). In a cross-sectional study, euglycaemic hyperinsulinaemic clamp (EHC) was performed in 83 non-diabetic subjects, neither glucose tolerance status nor insulin sensitivity both as measured using EHC and HOMA-IR, was found to be associated with serum vaspin levels (32). All these data indicate a controversy in the association of vaspin levels with insulin resistance. In our study, there was no association between vaspin levels and FPG, insulin, BMI or HOMA-IR values in hypothyroid patients. Glucose was reported to induce vaspin production and secretion from omental adipose tissue in a dose-dependent manner indicating that vaspin expression is induced by hyperglycaemia (29). Vaspin mRNA expression was found to be absent in lean normal glucose-tolerant individuals and was detected in type 2 diabetic patients (2). Only non-diabetic subjects were included in our study and this might be the cause of this lack of association. Moreover, the controversy in the association of vaspin with insulin levels may be due to different polymorphisms of serpins in different populations. Serpin genes represent a superfamily of proteins and ~500 serpins have been identified in a variety of species including animals, viruses and plants (33). Human α -1 antitrypsin is located within a cluster of serpin genes and has a wide spectrum of protein variants and shows different polymorphic frequencies in different human populations (34). Polymorphism of different proteins of serpin family was reported in many previous studies (35, 36). Therefore, we may speculate that the controversy in the association between vaspin levels and insulin resistance may be due to certain polymorphisms of serpins in our population. Our previous study also demonstrated similar vaspin levels in diabetic and non-diabetic subjects. Further investigation is needed to clarify this issue.

There is only one study about the regulation of vaspin by thyroid hormones on rats (14). Gonzalez *et al.* investigated the regulation of vaspin gene expression in rat white adipose tissue in various physiological and pathophysiological settings that are associated with energy homeostasis and insulin sensitivity. As disturbances in thyroid function alter insulin sensitivity and metabolic components, the authors investigated vaspin mRNA, glucose and insulin levels in hyperthyroid, euthyroid and hypothyroid rats. Vaspin mRNA levels were significantly downregulated in hyperthyroid rats and significantly increased in hypothyroid rats compared with euthyroid ones although no difference in insulin and glucose levels was observed (14). They

concluded that vaspin expression was affected by thyroid status in rats. In our study, vaspin levels were not correlated with TSH levels. Vaspin might be differently regulated in humans and rodents. Although it was not meant to investigate the relation of vaspin levels with thyroid functions, one study examined the relationship between TSH and vaspin and leptin levels before and after weight loss by bariatric surgery (15). The regulation of TSH by leptin and the effect of weight loss on thyrotropin levels in association with leptin levels were previously shown in humans (37). Handisurya *et al.* (15) showed that RYGB induced a significant reduction in circulating TSH levels, which positively correlated with changes in leptin and vaspin levels. The authors concluded that it is not known if the changes in vaspin levels are related to TSH alterations after weight loss or due to other confounding factors. Vaspin levels after bariatric surgery may be affected by differential changes in the secretion of gastrointestinal peptides. In our study, vaspin levels were not correlated with TSH levels and did not differ between hypothyroid and euthyroid subjects. Treatment of overt hypothyroid patients did not alter vaspin levels. Our study demonstrated that vaspin secretion was not regulated by TSH. The changes in vaspin levels after bariatric surgery may be primarily related to altered secretion of gastrointestinal peptides rather than TSH levels *per se* (15).

The main limitation of our study is the small sample size. Large-scale studies are therefore needed to identify the role of thyroid hormones on the regulation of vaspin.

In conclusion, vaspin levels did not seem to be associated with subclinical and clinical hypothyroid states. In our study cohort, hypothyroid patients have comparable insulin resistance parameters to control subjects that may explain the results of our study indicating similar vaspin levels in hypothyroid and euthyroid states. Moreover, establishment of euthyroidism by LT₄ treatment did not significantly change vaspin levels suggesting that euthyroid and hypothyroid states in the same patients have no influence on vaspin levels.

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 research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

Acknowledgements

We acknowledge the assistance of Metin Odevci during the performance of vaspin assays and Jale Karakaya for statistics.

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Received 23 February 2011

Revised version received 21 June 2011

Accepted 28 July 2011