

## ORIGINAL ARTICLE

# Increased leptin levels correlate with thyroid autoantibodies in nonobese males

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## Summary

**Objective** Leptin is an adipokine that regulates body weight and appetite. It is also an inflammatory cytokine that influences immune reactivity and autoimmunity. Leptin levels are increased in obesity and are higher in women than in men. We aimed to determine whether leptin levels, independent of sex and body mass index (BMI), are associated with thyroid autoimmunity.

**Design** This study uses data from The Third National Health and Nutrition Examination Survey (NHANES III) to test the association of leptin and thyroid autoimmunity, independent of BMI.

**Measurements** Thyroid-stimulating hormone, thyroxine, antithyroid peroxidase (TPO) antibodies and leptin levels were measured in 2902 men and 3280 women within the NHANES III population. BMI was calculated from height and weight.

**Results** Women had significantly higher leptin levels and anti-TPO antibody titres than men. Correlation analyses demonstrated that leptin levels were associated with anti-TPO antibody levels in the total population, but when men and women were analysed separately, this association was lost. We then stratified men and women into obese (BMI > 30) or nonobese (BMI ≤ 30) subgroups and determined the association between leptin levels and anti-TPO antibody titres for each subgroup. Using regression analysis, we found that increased leptin levels correlated with thyroid autoantibodies in nonobese males, but not in obese males or in females.

**Conclusions** Leptin levels correlated with thyroid autoantibody titres in nonobese males. This association was not found in females. Sex and body habitus should therefore be considered in studying the role of leptin in other autoimmune conditions.

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## Introduction

Leptin is a 16-kD hormone secreted from adipocytes in proportion to adipocyte mass and is therefore elevated in obesity. Leptin regulates body weight and appetite through signalling via leptin receptors that are highly expressed in the hypothalamus.<sup>1</sup> In addition to its role in regulating appetite and body weight, leptin also plays a key role in immunity as a pro-inflammatory cytokine.<sup>2</sup> Full-length leptin receptors are expressed on hematopoietic cells, including pro-inflammatory T-helper cells.<sup>3</sup>

Leptin deficiency in both mouse and human leads to immune cell defects characterized by decreased lymphocyte number, altered cytokine production and increased susceptibility to intracellular infections.<sup>4–7</sup> Additionally, multiple studies have identified a role for leptin in autoimmunity. Indeed, leptin-deficient (*ob/ob*) mice are protected against several autoimmune diseases, including experimental autoimmune encephalitis, systemic lupus erythematosus, colitis, glomerulonephritis and hepatitis.<sup>8,9</sup> Treatment of *ob/ob* mice with recombinant leptin reverses the protection against autoimmunity conferred by leptin deficiency. Likewise, in humans, elevated leptin levels are associated with higher rates of autoimmune disorders including multiple sclerosis, rheumatoid arthritis and type 1 diabetes.<sup>8</sup> Additionally, leptin accelerates the development of type 1 diabetes in the NOD mouse,<sup>9</sup> whereas conversely, leptin deficiency suppresses the development of diabetes in NOD mice. Lastly, fasting and calorie restriction, which result in weight loss and reduced leptin levels, have been shown to improve disease severity in select autoimmune disorders<sup>10</sup>; whereas obesity has been shown to increase the likelihood of developing autoimmunity.<sup>11–13</sup>

Autoimmune thyroid disease (Hashimoto's thyroiditis) is a polygenic disorder that results from a predisposed individual interacting with an environmental factor (e.g. infection, drugs and stress) that promotes the development of thyroid autoimmunity.<sup>14</sup> Autoimmune thyroiditis is the most common cause of acquired hypothyroidism.<sup>15</sup> Overt hypothyroidism has a prevalence of 0.8% in Americans, whereas subacute hypothyroidism occurs in 13% of Americans.<sup>16</sup> Moreover, women are 5–10 times more likely to develop autoimmune thyroid disease than men.<sup>17</sup> Autoimmune thyroiditis is characterized by immune destruction

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of the thyroid by both humoral (antibody) and cellular-mediated immune processes. Pro-inflammatory T-helper cells such as Th17 cells infiltrate the thyroid gland in Hashimoto's thyroiditis and may be included in the pathogenesis of the condition.<sup>18</sup> The hallmark to diagnosing autoimmune thyroiditis is the presence of antithyroid peroxidase (TPO) antibodies (also known as antimicrosomal antibodies) in the serum of hypothyroid patients.<sup>19</sup>

The relationship between thyroid function, leptin and body weight is complex.<sup>20,21</sup> Leptin influences thyroid hormone levels independent of any role in thyroid autoimmunity; leptin signalling promotes TRH secretion from the hypothalamus,<sup>22</sup> which then drives normal thyroid-stimulating hormone (TSH) secretion from the pituitary gland, resulting in thyroid hormone (thyroxine and triiodothyronine) production and secretion from the thyroid gland. Leptin can also promote spontaneous TSH secretion.<sup>23</sup> Elevated leptin levels have been observed in patients with Graves' disease taking beta-adrenergic receptor blockers, but not in those on antithyroid drugs.<sup>24</sup> Additionally, hypothyroidism oftentimes leads to weight gain and obesity, whereas obesity itself may alter TSH, thyroxine (T4) and triiodothyronine (T3) levels, with mild-to-moderate elevations in both TSH and T3, although reports on this vary.<sup>25,26</sup> Altogether, the interconnections between obesity, thyroid function and autoimmunity are complex and leptin may be a key factor to mediate these interactions.<sup>27</sup>

Circulating leptin levels are 2–3 times higher in women than in men.<sup>28</sup> Women also have increased risk of several forms of autoimmune disease in addition to thyroid autoimmunity, including multiple sclerosis, rheumatoid arthritis and systemic lupus erythematosus.<sup>29</sup> As leptin levels are increased in females, and in obesity, we evaluated the ability of leptin to correlate with thyroid autoimmunity in males vs females and in nonobese vs obese subjects. In this report, we analysed anti-TPO antibodies and leptin levels in 2902 men and 3280 women within The Third National Health and Nutrition Examination Survey (NHANES III) to test the association of leptin, thyroid autoimmunity and BMI.

## Methods

### Participants

We analysed data from the Third National Health and Nutrition Examination Survey (NHANES III). This population was selected to be generalizable to the civilian, noninstitutionalized US population, with an emphasis on the US Hispanic population.<sup>30, 31</sup> NHANES III was conducted between 1988 and 1994. A total of 39,695 people were selected for NHANES III, 33,994 underwent the interview, and 30,818 were examined by a physician. We were limited to a subgroup of NHANES III participants who had valid measurements of both serum leptin levels and anti-TPO antibodies drawn (6284 subjects). We excluded subjects who reported thyroid disease as well as any subject missing information regarding sex, BMI and age. This left us with 6182 patients for analysis: 2902 males and 3280 females.

### Laboratory assessments

Fasting venous samples were collected in the morning on NHANES III participants. Height and weight were measured by trained personnel. Body mass index (BMI) was calculated from these values and used to estimate obesity. In our study, we used a cut-off of BMI > 30 kg/m<sup>2</sup> to define our obese subgroups and BMI ≤ 30 kg/m<sup>2</sup> to define our nonobese subgroups. Leptin levels were analysed by radioimmunoassay using polyclonal rabbit anti-human antibodies. Assays were performed at Linco Research Inc., St. Louis, Missouri. The minimum detectable concentration of leptin was 0.5 µg/l leptin, with 100 µg/l as the upper limit of normal. Antimicrosomal/TPO antibody titres were used as a measure of thyroid autoimmunity. Anti-TPO antibodies were measured in NHANES III by radioimmunoassay and reported in international units (IU) (1 IU = 10 U/ml). Normal values (reference range) for anti-TPO antibodies in serum were less than 0.5 U/ml. For serum samples with anti-TPO antibody concentration >3000 after appropriate dilution, the final results were reported as >3000 U/ml.

### Statistical analysis

Continuous data were summarized using standard summary statistics (mean, standard deviation, median and interquartile range (IQR)), while categorical variables were presented using counts and percentages. Continuous variables were tested for normality using the Kolmogorov–Smirnov test, and either a *t*-test or Wilcoxon rank-sum test was used for comparisons, as deemed appropriate. Comparison of categorical data was performed using the chi-squared test or Fisher's exact test in the presence of small cell counts (<5).

Pearson correlation coefficients were used to analyse the association of leptin with anti-TPO antibodies in the total population of men and women combined and in subgroups of male and female patients. Male and female groups were further broken into obese and nonobese subgroups, and leptin association with anti-TPO antibodies was analysed in these subgroups using linear regression analyses. The multivariable models were sex specific. Each model included age, body mass index (BMI), leptin and the interaction of leptin and BMI.

A *P*-value < 0.05 was considered statistically significant, and all analyses were performed using SAS version 9.2 or higher.

## Results

### Comparison of men and women in our sample group

This study uses data from The Third National Health and Nutrition Examination Survey (NHANES III) to test the association of leptin and thyroid autoimmunity, independent of BMI. A total of 2902 men and 3280 women from NHANES III had anti-TPO antibodies and leptin levels drawn and were available for statistical analysis. The characteristics of male vs female participants are summarized in Table 1. The average age of the male participants was 47.8 ± 18.3 years, and the average age of

females was  $46.5 \pm 18.3$  years. Female participants had slightly higher mean BMI than males. As previously established, leptin levels were significantly higher in female than in male participants ( $P < 0.001$ ).<sup>32</sup> Although mean levels of TSH were slightly higher in females than in males, TSH measurements were not normally distributed and, therefore, any statistical difference is best examined through comparison of medians and interquartile ranges, which were not significantly different in males and females. Females had slightly but significantly higher mean thyroxine (T4) levels ( $P < 0.001$ ), likely secondary to changes in oestrogen- and thyroid-binding globulin levels. Additionally, females had significantly higher mean anti-TPO antibody levels than males; however, it is again important to note that anti-TPO levels were not normally distributed, and therefore, means and standard deviations for this measure were large due to extreme values in the patients with elevated titres. Median and interquartile ranges for anti-TPO antibody levels were identical, as the majority of subjects had antibodies in the nondetectable range.

### Correlation analysis for leptin and thyroid autoantibodies in men and women combined

Correlation analysis showed that leptin levels were highly associated with anti-TPO antibody levels in the total population of

**Table 1.** Descriptive variables by sex

Variable	Male	Female	P-value
Total number	2902	3280	
Age (years)			
Mean (SD)	47.8 (18.3)	46.5 (18.3)	0.004
Median (IQR)	45.0 (32.0–63.0)	42 (31.0–61.0)	
Race – number (%)			
Non-hispanic white	1206 (41.6)	1354 (41.3)	0.012
Non-hispanic black	760 (26.2)	954 (29.1)	
Hispanic	822 (28.3)	828 (25.2)	
Other	114 (3.9)	144 (4.4)	
Body mass index (BMI) (kg/m <sup>2</sup> )			
Mean (SD)	26.8 (4.8)	27.6 (6.4)	<0.001
Median (IQR)	26.2 (23.4–29.3)	26.4 (22.9–31.1)	
Serum leptin (ng/ml)			
Mean (SD)	6.0 (5.1)	17.9 (13.0)	<0.001
Median (IQR)	4.5 (2.8–7.5)	15.0 (8.9–23.5)	
Leptin quartiles – number (%)			
Low (<25 <sup>th</sup> %ile)	757 (26.1)	847 (25.8)	0.965
Normal (25 <sup>th</sup> –75 <sup>th</sup> %ile)	1421 (49.0)	1616 (49.3)	
High (>75 <sup>th</sup> %ile)	724 (25.0)	817 (24.9)	
Serum thyroxine (µg/dl)			
Mean (SD)	8.4 (1.9)	9.1 (2.2)	<0.001
Median (IQR)	8.4 (7.2–9.5)	8.9 (7.7–10.3)	
Serum thyroid-stimulating hormone TSH (µIU/ml)			
Mean (SD)	2.0 (7.8)	2.3 (4.9)	0.174
Median (IQR)	1.5 (1.0–2.2)	1.6 (1.0–2.4)	
Serum thyroid peroxidase (TPO) Ab (U/ml)			
Mean (SD)	4.2 (37.1)	12.2 (60.4)	<0.001
Median (IQR)	0.3 (0.3–0.3)	0.3 (0.3–0.3)	

male and female participants combined ( $r = 0.029$ ;  $P = 0.023$ ) (Table 2), but when male and female subjects were analysed separately, this association was lost ( $r = -0.004$ ;  $P = 0.821$  for males and  $r = -0.17$ ;  $P = 0.323$  for females). Given the strong possibility that obesity plays an important role in the association between leptin and thyroid autoimmunity, we decided to analyse and compare the obese vs nonobese populations in both male and female subjects.

### Analysis of obese vs nonobese subgroups in men and women

Comparison of obese and nonobese male subgroups showed no statistically significant differences in age ( $P = 0.367$ ), TSH levels ( $P = 0.061$ ) and thyroxine levels ( $P = 0.256$ ). Anti-TPO antibodies were not normally distributed, and median and interquartile ranges were identical, again because the majority of subjects had antibodies in the nondetectable range. There was a slight increase in percentage of Hispanic participants in the obese male subgroup as compared to the nonobese male subgroup, but overall differences in race were not statistically significant ( $P = 0.442$ ). As expected, obese males had statistically significant differences in BMI ( $P < 0.001$ ) and leptin levels ( $P < 0.001$ ) (Table 3a). Obese females were comparable in age ( $P = 0.896$ ) and had similar thyroxine levels ( $P = 0.492$ ) to nonobese females, but had significantly higher TSH ( $P = 0.004$ ). While obese females had slightly statistically different mean anti-TPO antibody titres ( $P = 0.047$ ), the median and interquartile ranges were identical, and more appropriate to examine as anti-TPO antibody levels were not normally distributed. There was again an increase in percentage of Hispanic participants in the obese compared to the nonobese female subgroup and significant differences in race ( $P < 0.001$ ), BMI ( $P < 0.001$ ) and leptin levels ( $P < 0.001$ ) (Table 3b).

Regression analyses of males and females stratified into obese (BMI > 30) vs nonobese (BMI ≤ 30) subgroups showed that increased leptin levels positively correlate with thyroid autoantibodies in nonobese males after adjusting for BMI and age ( $P = 0.021$ ). This relationship was not present in obese males or in females (Table 4). When we evaluated the interaction of leptin and BMI on thyroid autoantibody levels, we found that as BMI increases, the effect of leptin on thyroid autoantibody levels decreases (Table 4). These results imply that both obesity and female sex are stronger factors in developing thyroid autoimmunity than increased leptin levels.

**Table 2.** Correlation of leptin and anti-TPO antibody titres

	All subjects (male and female)	Male	Female
Pearson correlation coefficient	0.029	-0.004	-0.017
P-value	0.023	0.821	0.323

**Table 3a.** Obese vs nonobese male subjects

Variable	Nonobese male (BMI ≤ 30)	Obese male (BMI > 30)	P-value
Total number	2309	593	
Age (years)			
Mean (SD)	47.7 (18.8)	48.5 (16.2)	0.367
Median (IQR)	45.0 (32.0–63.0)	47.0 (35.0–62.0)	
Race – number (%)			
Non-hispanic white	970 (42.0)	236 (39.8)	0.442
Non-hispanic black	602 (26.1)	158 (26.6)	
Hispanic	642 (27.8)	180 (30.4)	
Other	95 (4.1)	19 (3.2)	
Body mass index (BMI) (kg/m <sup>2</sup> )			
Mean (SD)	24.9 (2.9)	33.9 (4.0)	<0.001
Median (IQR)	25.1 (22.8–27.3)	32.7 (31.2–35.2)	
Serum leptin (ng/ml)			
Mean (SD)	4.6 (3.5)	11.5 (6.5)	0.001
Median (IQR)	3.8 (2.5–5.9)	10.0 (7.2–14.2)	
Leptin quartiles – number (%)			
Low (<25th %ile)	747 (32.4)	10 (1.7)	<0.001
Normal (25th–75th %ile)	1264 (54.7)	157 (26.5)	
High (>75th %ile)	298 (12.9)	426 (71.8)	
Serum thyroxine (µg/dl)			
Mean (SD)	8.4 (1.9)	8.3 (1.9)	0.256
Median (IQR)	8.4 (7.3–9.5)	8.4 (7.0–9.6)	
Serum thyroid-stimulating hormone TSH (µIU/ml)			
Mean (SD)	1.9 (3.4)	2.6 (15.8)	0.061
Median (IQR)	1.4 (1.0–2.2)	1.6 (1.1–2.3)	
Serum thyroid peroxidase (TPO) Ab (U/ml)			
Mean (SD)	4.4 (40.2)	3.5 (21.1)	0.581
Median (IQR)	0.3 (0.3–0.3)	0.3 (0.3–0.3)	

**Table 3b.** Obese vs nonobese female subjects

Variable	Nonobese female (BMI ≤ 30)	Obese female (BMI > 30)	P-value
Total number	2308	965	
Age (years)			
Mean (SD)	46.4 (18.9)	46.5 (16.8)	0.896
Median (IQR)	42.0 (31.0–61.0)	43.0 (33.0–59.0)	
Race – number (%)			
Non-hispanic white	1052 (45.6)	301 (31.2)	<0.001
Non-hispanic black	592 (25.6)	361 (37.4)	
Hispanic	552 (23.9)	271 (28.1)	
Other	112 (4.8)	32 (3.3)	
Body mass index (BMI) (kg/m <sup>2</sup> )			
Mean (SD)	24.3 (3.3)	35.5 (5.0)	<0.001
Median (IQR)	24.4 (21.7–26.8)	34.1 (31.7–37.7)	
Serum leptin (ng/ml)			
Mean (SD)	13.1 (8.1)	29.5 (15.1)	<0.001
Median (IQR)	11.6 (7.4–17.0)	26.8 (20.1–35.9)	
Leptin quartiles – number (%)			
Low (<25th %ile)	820 (35.5)	26 (2.7)	<0.001
Normal (25th–75th %ile)	1 273 (55.2)	339 (35.1)	
High (>75th %ile)	215 (9.3)	600 (62.2)	
Serum thyroxine (µg/dl)			
Mean (SD)	9.1 (2.2)	9.0 (2.2)	0.492
Median (IQR)	9.0 (7.7–10.4)	8.8 (7.7–10.2)	
Serum thyroid-stimulating hormone TSH (µIU/ml)			
Mean (SD)	2.1 (3.5)	2.6 (7.3)	0.004
Median (IQR)	1.5 (1.0–2.3)	1.7 (1.2–2.6)	
Serum thyroid peroxidase (TPO) Ab (U/ml)			
Mean (SD)	11.2 (62.2)	17.7 (118.8)	0.047
Median (IQR)	0.3 (0.3–0.3)	0.3 (0.3–0.3)	

## Discussion

We found that leptin levels were associated with anti-TPO antibody levels in the total population of men and women combined, but when men and women were analysed separately, this association was lost. Regression analyses of men and women stratified into obese (BMI > 30) or nonobese (BMI ≤ 30) subgroups showed that increased leptin levels correlate with thyroid autoantibodies in nonobese males, but not in obese males or in females. These findings underscore the influence of sex and obesity on autoimmunity<sup>33</sup> and highlight sex differences in the potential role of leptin in promoting autoimmune thyroid disease.

The loss of a positive association between leptin and anti-TPO antibodies when men and women were analysed separately (Table 2) is an example of Simpson's paradox. This paradox occurs when an association observed in different groups (male and female groups in our case) is different than when the groups are combined. We believed this occurred in our study because of the overwhelming effect that sex had on the level of antithyroid antibody levels.

A previous case-control study by Marzullo *et al.* published in 2010 compared TSH, anti-TPO antibodies and leptin levels

in 165 obese (BMI ≥ 35) and 118 lean subjects (BMI ≤ 25).<sup>34</sup> In this report, leptin was associated with thyroid autoimmunity independent of obesity/BMI. This study, however, did not separate male and female subjects for analysis. Our study also shows that leptin levels were associated with thyroid autoimmunity when we looked at all subjects, male and female, combined. But given the significant difference in leptin levels between male and female subjects (Table 1), we chose to examine the effect of leptin on thyroid autoantibodies in male and female populations separately. These results were very surprising, as we lost any association of leptin with thyroid autoantibody levels in the separated male and female populations. Indeed, it was only upon stratifying male and female subjects into obese vs nonobese subgroups that we were able to discern the correlation of leptin with thyroid autoantibodies in nonobese males. These findings underscore the importance of examining male and female groups separately, a point that was recently made by Woodruff *et al.* in a commentary published earlier this year in *Endocrinology*.<sup>35</sup> Moreover, our results show that both obesity and female sex are stronger factors in developing thyroid autoimmunity than leptin levels. Other adipokines may be more important than leptin in promoting autoimmune thyroiditis.

**Table 4.** Multivariable regression model for TPO antibody (U/ml) among sex and BMI subgroups. Results shown as parameter estimate (*P*-value)

Parameter	Female		Male	
	BMI ≤ 30	BMI > 30	BMI ≤ 30	BMI > 30
Interaction leptin*BMI	-0.03 (0.399)	0.02 (0.222)	-0.14 (0.017)	-0.01 (0.724)
Leptin (µg/l)	0.47 (0.638)	-1.03 (0.144)	3.60 (0.021)	0.04 (0.958)
BMI (kg/m <sup>2</sup> )	0.63 (0.334)	-0.56 (0.458)	0.64 (0.056)	1.08 (0.129)
AGE (per 10 years)	2.15 (0.004)	2.94 (0.047)	0.76 (-0.097)	0.02 (0.969)

Our study has several strengths. Ours is the first population-based study of the relationship between leptin and autoimmunity of any kind. The study sample was very large. Anthropomorphic measurements were accurate, and clinical subgroups were used to study associations. Limitations to our study were inadequate information about the diagnosis of hypothyroidism in subjects, about menopause in women and about the use of birth control pills; these factors could be confounders, as sex hormone levels could contribute to changes in thyroid hormone. Moreover, the use of anti-TPO antibodies as a marker of autoimmune thyroid disease is not ideal, as thyroid autoantibodies can be detected in euthyroid patients; however, current evidence suggests that positive thyroid autoantibodies in euthyroid patients may predict subsequent hypothyroidism.<sup>36–38</sup>

The implications of our study are the following. First, animal experiments pertaining to potential immune effects of leptin and other adipokines should be performed in both male and female animals. Next, analyses of the effect of leptin and other autoimmune conditions should be performed in subset by sex and obesity, as in our study. Last, the pathogenesis of thyroid autoimmunity is complex and likely influenced by an array of hormones, cytokines, metabolites and genetic factors.

### Conflict of interest

Nothing to declare.

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