Association of Serum Thyroid Hormones and Serum Leptin with Body Mass Index

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A R T I C L E   I N F O

Article History:
Received 03 October 2013
Received in revised form 06 October 2013
Accepted 12 October 2013
Available online 25 October 2013

Key words:
Leptin, BMI, Thyroid hormones, Pakistan.

A B S T R A C T

Leptin is a hormone secreted by adipose tissues and is considered to be a satiety hormone, which inhibits feeding. Much research is being carried out internationally to understand role of leptin in obesity and related disorders. Thyroid hormones are important in metabolism and energy expenditure. Very few researches of serum leptin and thyroid hormones with body mass index in Pakistan. Objective of this study was to find any possible association of serum leptin levels and thyroid hormones, namely, thyroxine (T4), triiodothyronine (T3) and thyroid stimulating hormones (TSH) with body mass index (BMI). This prospective, randomized study was conducted in Lahore, Pakistan. The subjects were selected from Outpatient Department of Internal Medicine, Mayo Hospital and British Slimming Center.

Introduction

Leptin has a key role in body weight regulation by serving as an adipose satiety signal to the brain thereby influencing food intake and energy expenditure in a negative feedback loop (Huang and Li, 2000). Leptin mRNA and leptin secretion in adipocytes have been shown to be modulated by thyroid hormones (Yoshida et al., 1997). TSH stimulates leptin secretion by human adipose tissue in vitro (Menendez et al., 2003). In fed state, leptin has an acute stimulatory effect on TSH release in vivo, acting probably at the hypothalamus, however, the direct pituitary effect of leptin is inhibitory (Ortiga-Carvalho et al., 2002). This suggests that leptin and thyroid axis have some relationship.

It is well known for decades that thyroid hormones (TH) play a key role in regulating energy homeostasis (Yen, 2001), the obesity pandemic has driven new interest in the relationship between TH and weight status. Weight loss is a typical sign of thyroid hyperfunction, whereas hypothyroidism is generally associated with weight excess (Reinehr, 2010). The biological mechanisms responsible for the effects of leptin in stimulating energy expenditure are still far from being resolved. Because thyroid hormone plays a fundamental role in regulation of energy metabolism, it is of great interest to explore the interrelationship between leptin and thyroid hormones. The relationship between weight and Thyroid hormones has not been scientifically assessed. Therefore, practitioners commonly deal with overweight patients who believe that small changes in thyroid function have significant impact on body composition. In such patients obesity may be related to thyroid hormones (Weiss and Brown, 2008). There is a universal agreement that thyroid function is initially determined by serum thyrotropin (TSH) concentration. Unfortunately, the agreement is lacking as regards the definition of normal thyroid function as such (Brabant et al., 2006; Dayan et al., 2002; Galofre et al., 2006; Surks et al., 2005). A specific TSH normal range for different situations is needed, including ethnic origin, age sex, health status and, probably, body mass index (BMI) (Fatourechi, 2007). Wide TSH level variations are common when serum samples from different healthy subjects are compared,
even if the analysis is performed within the same age range. For instance, there is compelling evidence that normal TSH levels increase with age, although a recent study found that TSH secretion is gender invariant and depends on age in women only (Roelfsema et al., 2009).

More than a century has elapsed since the first clinical observation that hyperthyroid patients tend to lose weight and the reversibility of this tendency once treatment is established (Silva, 2010). However, unfortunately the explanation for this behavior is still elusive in many aspects. Epidemiological data generally show a higher prevalence of overt and subclinical hypothyroidism (~20%) in morbid obese individuals (Michalaki et al., 2005). Many groups have reported that baseline serum TSH levels are usually in the upper limit (or slightly over it) of the normal range in euthyroid obese individuals (Iacobellis et al., 2005). Although the range of Thyroid hormones values may vary in different populations (regarding, for instance, dietary iodine intake or other factors), the usual finding is that TSH levels correlate with body weight (Iacobellis et al., Moulin de Moraes et al., 2005). Additionally, in these subjects (even in euthyroid individuals), the increase in TSH concentrations is associated with elevated waist circumference and BMI (Fox et al., 2008; Waterhouse et al., 2007), which is a non-consistent finding as several studies found that TSH secretion is gender invariant and, this situation seems to be metabolic alterations related with obesity may regulate thyroid homeostasis; and, this situation seems to be Extreme longevity has been associated with an increase in serum TSH concentrations (Atzmon et al., 2009) with thyroid diseases being more prevalent in older people (Flynn et al., 2004). However, it is not known whether the increase in serum TSH levels represents a physiological trend related to aging or the consequence of illness (Mariotti, 2005). Therefore, an elevation in serum TSH level may be the translation of two situations with opposite influence on longevity (Mariotti, 2008). A low metabolic rate (which is associated with high TSH level) is a longevity marker. Furthermore, caloric restriction slows down the aging process. On the other hand, illness is a known cause of elevation in TSH – there are a good number of pathological conditions associated with mild or subclinical hypothyroidism (Biondi and Cooper, 2008; Heilbronn et al., 2006). Thus, mild hypothyroidism seems to be detrimental for young or middle aged subjects, whereas it may be harmless or perhaps beneficial for advanced aged individuals (Mariotti, 2008). Therefore, it looks as if some of the metabolic alterations related with obesity may regulate thyroid homeostasis; and, this situation seems to be

### Table-1: Statistical Analysis of Thyroid Hormones

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Statistical Significance</th>
<th>serum leptin (ng/ml)</th>
<th>T4 (ng/dl)</th>
<th>T3 (pg/ml)</th>
<th>TSH (µIU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI (Wt in Kg/ ht in m2)</td>
<td>Correlation</td>
<td>.316(**)</td>
<td>.277(**)</td>
<td>.250(*)</td>
<td>.452(**)</td>
</tr>
<tr>
<td></td>
<td>p-value</td>
<td>.002</td>
<td>.008</td>
<td>.017</td>
<td>.000</td>
</tr>
<tr>
<td>serum leptin (ng/ml)</td>
<td>Correlation</td>
<td>.113</td>
<td>.171</td>
<td>.276(**)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>p-value</td>
<td>.285</td>
<td>.105</td>
<td>.009</td>
<td></td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>Correlation</td>
<td>.125</td>
<td>.127</td>
<td>.189</td>
<td></td>
</tr>
<tr>
<td></td>
<td>p-value</td>
<td>.238</td>
<td>.230</td>
<td>.075</td>
<td></td>
</tr>
<tr>
<td>T-Chol (mg/dl)</td>
<td>Correlation</td>
<td>.242(*)</td>
<td>.150</td>
<td>.262(*)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>p-value</td>
<td>.021</td>
<td>.155</td>
<td>.013</td>
<td></td>
</tr>
<tr>
<td>HDL-C (mg/dl)</td>
<td>Correlation</td>
<td>.186</td>
<td>.011</td>
<td>.047</td>
<td></td>
</tr>
<tr>
<td></td>
<td>p-value</td>
<td>.078</td>
<td>.915</td>
<td>.664</td>
<td></td>
</tr>
<tr>
<td>LDL-C (mg/dl)</td>
<td>Correlation</td>
<td>.030</td>
<td>.170</td>
<td>.015</td>
<td></td>
</tr>
<tr>
<td></td>
<td>p-value</td>
<td>.777</td>
<td>.107</td>
<td>.889</td>
<td></td>
</tr>
<tr>
<td>LDL-C/HDL-C ratio</td>
<td>Correlation</td>
<td>-.016</td>
<td>.165</td>
<td>-.017</td>
<td></td>
</tr>
<tr>
<td></td>
<td>p-value</td>
<td>.879</td>
<td>.119</td>
<td>.871</td>
<td></td>
</tr>
<tr>
<td>T4 (ng/dl)</td>
<td>Correlation</td>
<td>-.031</td>
<td>.027</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>p-value</td>
<td>.770</td>
<td>.798</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T3 (pg/ml)</td>
<td>Correlation</td>
<td>.171</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>p-value</td>
<td></td>
<td>.109</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The observed positive association between TSH and BMI could be due to alterations in Thyroid hormones activity or as a result of an alteration in the regulation of the hypothalamic-pituitary-thyroid (HPT) axis. The hypothesis that involves a direct effect of TSH is also plausible as the TSH receptor is expressed in adipose tissue (Peeters et al., 2007). It has been published that circulating cytokines related with metabolic syndrome can suppress thyroid function either at hypothalamic or pituitary or thyroid levels. The more suitable contributing factor is the deregulation of the HPT axis in the obese population, since a direct relationship between TSH and BMI has been consistently observed (Sari et al., 2003). However, as aforementioned, there are conflicting data in the literature regarding the relationship between obesity and Thyroid hormones levels. Some studies, but not all, demonstrated low T3 and low T4 at higher body weight and BMI levels, whereas other authors found a direct relationship between free T3 and BMI. Therefore, there are a number of factors that contribute to free T3 levels in obese subjects. These factors could vary among different subjects with same BMI, like body composition, underlying thyroid diseases, iodine intake, etc.

Studies in rodents have shown a dramatic down-regulation of TRH gene expression in the paraventricular nucleus (PVN) during fasting. Direct and indirect effects of decreased serum leptin, in addition to effects on increased local T3 concentrations in the hypothalamus during food deprivation, contribute to a decreased activity of TRH neurons in the PVN. Pituitary TSH mRNA expression also decreases during fasting, and this may be relatively independent of leptin and/or TRH, since leptin administration in this setting does not fully restore pituitary TSH expression, while it does restore TRH expression in the PVN. The observed decrease in serum Thyroid hormones concentrations is the result to some extent of a diminished thyroidal secretion of Thyroid hormones. The overall result of these complex HPT axis changes in various tissues during fasting is down-regulation of the HPT axis, which is assumed to represent an energy-saving mechanism, instrumental in times of food shortage (Boelen et al., 2008). Thus, it seems that the adipocyte-derived hormone, leptin, may be at the origin of this dysfunction (Mantzoros et al., 1997), although other possibilities may also exist. Some investigators have suggested the existence of partially bio-inactive TSH in obese subjects, although this hypothesis is very speculative. Other authors suggest that there may be certain TH resistance, as well as decreased T3 receptors in obese subjects (Burman et al., 1980).

Materials and methods

In the present study, 100 subjects (50 obese and 50 non obese) were selected from the Outpatient Department of Internal Medicine, Mayo Hospital Lahore and British Sliming Center, Lahore, Pakistan. Ethical approval was obtained from the local institution’s review committee and consent was obtained from all participants. For the purposes of this study, subjects were classified into two categories; Subjects with obesity, BMI>30 and subjects without obesity, BMI<25. Subjects taking part in research did not have diabetes mellitus, hypertension, cardiovascular disease or any malignancies, hepatitis B or any contagious disease.

Analytical method

The nutritional status of all subjects was assessed by means of anthropometric measurements. The body weight of each individual dressed in light clothing was measured using a carefully calibrated weighing balance. The height of each individual was measured using a vertical-measuring rod; waist and hip circumferences were also measured to calculate waist/hip ratio. BMI was calculated as weight in kg divided by squared height (m²). Blood samples were taken early in the morning, 12 hours postprandial. About 10 ml of venous blood was drawn from the subjects. The serum samples were stored at 2-5°C for not more than 24 hours prior to serum leptin and thyroid hormones (T3, T4 & TSH) determination.

Laboratory techniques

1. Leptin measurement through ELISA
   Human leptin ELISA kit (ENZO-ALX-850-044-KI01) was used (that is designed with sandwich enzyme immunoassay method for the quantitative measurement of human leptin).

2. Thyroid Hormones
   i. Tri-iodothyronine (T3) Concentration:
      Serum T3 concentration was measured by using ELISA (Coretiz Diagnostic Inc.) with Detection Range of 0-10ng/ml, Specificity 97.50% and Sensitivity 0.2 ng/mL
   ii. Thyroxine (T4) Concentration:
       Serum T4 concentration was measured by using ELISA (Coretiz Diagnostic Inc.) with Detection Range of 0-30ng/ml , Specificity 96.30% and Sensitivity 0.5 mg/dl
   iii. Thyroid Stimulating Hormone (TSH) Concentration:
        Serum TSH concentration was measured by using ELISA (Coretiz Diagnostic Inc.) with Specificity was 97.10% and Sensitivity 2 µIU/mL

Statistical analysis

Results were presented as the mean +/- standard deviation. Data was analyzed using the Statistical Package for the Social Sciences (SPSS) version 16 (SPSS, Evanston, IL, USA). Biochemical parameters not normally distributed were analyzed after being logarithmically transformed. Students’ unpaired t-test and one-way analysis of variance (ANOVA) were used to compare the results of the different groups. Simple and partial correlation coefficients between the variables were determined and multiple regression analysis was performed to determine the relationships between the variables of interest. Data was expressed as mean (SD) or median (range); statistical significance was set at p < 0.05.

Discussion

Although much has been learnt regarding the leptin hormone, its physiology and the precise role it plays in the endocrine system remain to be defined. One of the difficulties inherent to these studies lies in the fact that leptin physiology seems to be rather different in humans and rodents. Not only is the circadian rhythm of its plasma levels different but also its regulation and the relationship with other hormones have been shown to differ. Recently, some studies in rats have...
demonstrated a negative influence of thyroid hormones on leptin levels, independently of the changes in body weight due to the thyroidal effect (Escobar-Morreale et al., 1997). This study reveals that leptin levels are positively correlated with thyroid hormones i.e., T\textsubscript{3}, T\textsubscript{4}, and TSH.

Our prospective analysis is consistent with a major effect of thyroid hormones on leptin levels, as leptin levels change with the change in BMI. This suggests that a plasma leptin concentration in humans reflects the changes in energy expenditure due to the thyroidal effect.

In summary, while inhibition of serum leptin by thyroid hormones has been demonstrated in rodents treated with both T\textsubscript{3} and T\textsubscript{4}, in humans greater BMI results in lower levels of thyroid hormones and high levels of Leptin.

An association between TSH and leptin levels has been reported previously. It has been suggested that leptin stimulates TRH release from the hypothalamus, directly or indirectly, which in turn stimulates TSH secretion, while the direct pituitary effect of leptin on TSH release is inhibitory, as shown by the in vitro results. Therefore, it can be suggested that with a chronic and higher degree of hyperthyroidism leptin would not be able to change TSH release because of the profound effects of thyroid hormone reducing not only the release but mostly the synthesis of TRH and TSH. Serum leptin levels showed significant correlation with the BMI in groups which is consistent with several studies.

Conclusion
It is concluded that as thyroid hormones have direct effects on energy expenditure and body weight, they are positively related with the circulating serum leptin levels. Thyroid hormones directly affect basal metabolic rate, appetite and body weight. The mechanisms responsible for the production of the physiological effect of thyroid hormones on weight and energy homeostasis are complex and have not yet been fully elucidated. In the present study, significantly high levels of serum leptin have been observed in obese patients. As the subjects included in this study are not adequate, a comprehensive study must be conducted to fully understand the relationship of BMI to thyroid hormones and leptin levels.

Acknowledgement
We wish to extend our deep gratitude for the administration of the University of Central Punjab (UCP) for their support and providing facilities to make this article possible. We are especially thankful to Prof. Dr. Muhammad Jamshaid, Dean Faculty of Pharmacy for his guidance and cooperation. Last but not the least we express our appreciation and thanks to Prof. Dr. Muhammad Zafarullah for his continued help in
developing a culture of research in the University.

References


Dayan CM, Saravanan P, and Bayly G (2002), 'Whose normal thyroid function is better—yours or mine?', Lancet, 360 (9330), 353.


Flynn RW, et al., (2004), 'The thyroid epidemiology, audit, and research study: thyroid dysfunction in the general population', J Clin Endocrinol Metab, 89 (8), 3879-84.


Manji N, et al., (2006), 'Lack of association between serum TSH or free T4 and body mass index in euthyroid subjects', Clin Endocrinol (Oxf), 64 (2), 125-8.


Mariotti S (2005), 'Thyroid function and aging: do serum 3,5,3'-triiodothyronine and thyroid-stimulating hormone concentrations give the Janus response?', J Clin Endocrinol Metab, 90 (12), 6735-7.

Mariotti S (2008), 'Mild hypothyroidism and ischemic heart disease: is age the answer?', J Clin Endocrinol Metab, 93 (8), 2969-71.


Michalaki MA, et al., (2006), 'Thyroid function in humans with morbid obesity', Thyroid, 16 (1), 73-8.


Roelfsema F, et al., (2009), 'Thyrotropin secretion profiles are not different in men and women', J Clin Endocrinol Metab, 94 (10), 3964-7.


Silva JE (2010), 'Fat and energy economy in hypo- and hyperthyroidism are not the mirror image of one another', Endocrinology, 151 (1), 4-6.

Surks MI, Goswami, G., and Daniels, G. H. (2005), 'The thyrotropin reference range should remain unchanged', J Clin Endocrinol Metab, 90 (9), 5489-96.


Weiss RE and Brown RL (2008), 'Doctor . . . could it be my thyroid?', Arch Intern Med, 168 (6), 568-9.

Yen PM (2001), 'Physiological and molecular basis of thyroid hormone action', Physiol Rev, 81 (3), 1097-142.