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ORIGINAL PAPER

Possible implications of leptin, adiponectin and ghrelin in the regulation of energy homeostasis by thyroid hormone

Alexander Kokkinos · Iordanis Mourouzis · Despoina Kyriaki · Constantinos Pantos · Nicholas Katsilambros · Dennis V. Cokkinos

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Abstract Thyroid hormone plays a critical role in energy homeostasis through mechanisms, which are not fully understood. In the present study, we investigated possible alterations of important energy regulators such as leptin, adiponectin, and ghrelin in relation to changes in thyroid hormones. Thyroid hormone (250 μg/kg) was administered in male Wistar rats for 2 weeks (THYR), while hypothyroidism (HYPO) was induced by propylthiouracil administration (0.05% in drinking water) for 3 weeks. Untreated animals served as controls (NORM). Leptin and adiponectin were measured in plasma by ELISA, while total ghrelin was measured with RIA. Body weight was significantly reduced both in THYR and HYPO rats, while food intake was significantly increased in THYR and decreased in HYPO. This response was associated with various changes in leptin, adiponectin, and ghrelin in plasma. In fact, in THYR rats, leptin levels (mean ± SEM) were 240 ± 55 pg/ml as compared to 819 ± 70 pg/ml in untreated rats ($P < 0.05$), while no changes were observed in ghrelin and adiponectin. In HYPO rats, leptin levels were 1400 ± 200 pg/ml vs. 819 ± 70 pg/ml in untreated rats ($P < 0.05$), while ghrelin and adiponectin were significantly increased in HYPO rats as compared to untreated rats ($P < 0.05$). Furthermore, T_3 and T_4 levels were inversely correlated to leptin ($P = 0.014$), while ghrelin and adiponectin were inversely correlated to weight changes ($P = 0.05$ and $P = 0.03$, respectively). In conclusion, leptin seems mainly to be involved in the thyroid hormone effects on energy homeostasis. Ghrelin and adiponectin may serve a compensatory physiological role in hypothyroidism.

Keywords Thyroid hormone · Leptin · Adiponectin · Ghrelin · Food intake · Body weight

A. Kokkinos · D. Kyriaki · N. Katsilambros
1st Department of Propaedeutic Medicine, Laiko General Hospital, University of Athens Medical School, 11527 Goudi, Athens, Greece

I. Mourouzis · C. Pantos (✉)
Department of Pharmacology, University of Athens, 75 Mikras Asias Ave., 11527 Goudi, Athens, Greece
e-mail: cpantos@cc.uoa.gr

D. V. Cokkinos
1st Cardiology Department, Onassis Cardiac Surgery Center, 356 Sygrou Ave., 17674 Kallithea, Athens, Greece

Introduction

Thyroid hormones play an important role in energy homeostasis by regulating energy intake and expenditure. Accordingly, variations in food intake and body weight are seen in conditions associated with thyroid hormone abnormalities. However, although it has long been recognized that thyroid hormones increase food intake and reduce body weight gain, little is known regarding the possible underlying mechanisms. Recent research provides new evidence showing that adipokines, such as leptin and adiponectin have a role in energy homeostasis [1]. Leptin, at least in rodents, acts on hypothalamic appetite center to limit the caloric surplus to fit the available adipocyte storage capacity [2]. Adiponectin has been shown to increase energy metabolism, induce fatty acid oxidation, and promote mitochondrial biogenesis [1, 3]. Both leptin and adiponectin have been shown to change in states of thyroid dysfunction [4]. Gastrointestinal hormones, such as ghrelin may also have a critical role in the regulation of energy homeostasis, and is shown to stimulate food intake and gastric emptying in rats [5]. On the basis of this

evidence, it could be hypothesized that these signaling pathways may serve an important role in the regulation of energy homeostasis by thyroid hormones. So far, this issue has not been adequately explored. In fact, findings between studies in humans and in laboratory animals have been conflicting. Therefore, in the present study, we investigated whether the effects of thyroid hormones directly or through their influence on energy homeostasis, as assessed by food intake and body weight changes, are associated with changes in leptin, adiponectin, and ghrelin levels in plasma. This may be of physiological and therapeutic relevance. Thyroid analogs are now suggested to be potential therapeutic agents for controlling body weight and lipid metabolism [6, 7].

Methods

Eighteen male Wistar rats (8–12 weeks old) were used for this study. All the procedures were carried out in accordance with the Guide for the Care and Use of Laboratory Animals, published by the US National Institutes of Health Guide (NIH Pub. No. 83-23, Revised 1996). L-thyroxine (250 μg/kg, Sigma Chemicals, St. Louis MO, USA) was administered sc, once daily, for 14 days (THYR, $n = 6$), while propylthiouracil (0.05%) was administered in drinking water for 21 days (HYPO, $n = 6$). Untreated male animals served as controls (NORM, $n = 6$). Rats were housed in individual cages for the last 48 h of treatment, and food intake (in g) was determined during this period. A fixed quantity of diet, containing 3% lipid, 20% protein, and 77% carbohydrate, was supplied in each cage. Body weight was measured at the beginning and at the end of treatment, and the change in body weight (ΔBw in g) was measured for each group.

Plasma L-thyroxine and 3,5,3′tri-iodothyronine quantitative measurements were performed with ELISA, using kits obtained from Alpha Diagnostic International, Texas, USA (No. 1100 for total T_4 and No. 1700 for total T_3), as previously described [8]. L-thyroxine and 3,5,3′ tri-iodothyronine levels were expressed as nmol/l of plasma. Absorbance measurements were performed at 450 nm with Tecan Genios ELISA reader (Tecan, Austria).

Adiponectin (in μg/ml), and leptin (in pg/ml) were measured in plasma using ELISA kits (Linco Research, EZRADP-62K for rat adiponectin and R&D Systems, MOB00 for rat leptin). Total ghrelin (in pg/ml) was measured in serum by RIA kit (Linco Research, GHRT-89HK for rat ghrelin). Plasma and serum collection was performed according to manufacturers' guidelines.

Inter-Assay variations for each hormone determined in the present study were 7–9% for total T_3, 8–10% for L-thyroxine, 3.3–4.3% for leptin, 1–2% for adiponectin, and 6–10% for ghrelin.

Results are presented as mean (SEM). One-way analysis of variance with Bonferonni or Dunnett's correction was used to evaluate differences between groups. Multiple correlations were performed by calculation of correlation coefficient and controlling for inter-relationships between factors, which potentially control body weight. Significance was set at 0.05.

Results and discussion

Thyroxine administration in rats for 2 weeks resulted in increase in food intake accompanied by lower levels of leptin in plasma (Table 1). Since leptin has previously been shown to be involved in the regulation of food intake in rats [2], this finding may indicate that the thyroid hormone effect on food intake may be, at least in part, mediated by changes in plasma leptin levels. This response is probably due to either a direct effect of thyroid hormone on leptin regulation or to weight changes induced by thyroid hormone. The latter seems unlikely, since in our model of hypothyroidism, in which a significant weight reduction was observed, leptin levels in plasma were significantly increased (Table 1). The first possibility is more likely and appears to be supported by our observation that leptin levels and food intake were negatively correlated in Table 2. Our finding seems to be in accordance with other reports showing decreased leptin levels in hyper- and increased leptin levels, in hypo-thyroid rats [9]. Studies in humans also show similar results. Ishii et al. actually postulate that decreased leptin may contribute to hyperphagia in T_3 induced thyrotoxicosis [10].

There is now evidence that thyroid hormone can negatively regulate leptin gene expression [11]. Interestingly, a negative correlation between the levels of T_3, T_4, and leptin

Table 1 T_4 and T_3 levels in plasma, food intake (g) during the last 48 h of treatment, body weight change (ΔBz), leptin, ghrelin, and adiponectin levels in hyperthyroid (THYR), hypothyroid (HYPO), and untreated rats (NORM). Data are presented as mean (SEM)

	NORM	THYR	HYPO
T_4 (nM)	62.6 (4.7)	716 (39)*	18.5 (2.5)**
T_3 (nM)	1.1 (0.2)	8.9 (1.0)*	0.38 (0.1)**
Food Intake (g)	54 (1.7)	69 (2.1)*	31 (2.3)**
ΔBw (g)	+20 (4)	+5 (2)*	–8 (4)*
Leptin (pg/ml)	819 (70)	240 (55)*	1,400 (200)**
Ghrelin (pg/ml)	1,940 (185)	2,600 (500)	10,365 (700)**
Adiponectin (μg/ml)	6.63 (0.6)	7.5 (0.5)	22.2 (1.4)**

* $P < 0.05$ vs. NORM, ** $P < 0.05$ vs. NORM and THYR

Table 2 Multiple correlations performed between T_4 and T_3 levels in plasma, change in body weight (ΔBz), food intake and leptin, ghrelin, and adiponectin levels. Correlation coefficient (r) and statistical significance (P) are indicated

Multiple Correlations	
Leptin vs. T_3	r = –0.62, P = 0.014
Leptin vs. T_4	r = –0.61, P = 0.014
Leptin vs. ΔBw	r = 0.07, P = n.s.
Leptin vs. Food Intake	r = –0.7, P = 0.004
Ghrelin vs. T_3	r = –0.01, P = n.s.
Ghrelin vs. T_4	r = –0.001, P = n.s.
Ghrelin vs. ΔBw	r = –0.4, P = 0.05
Ghrelin vs. Food Intake	r = –0.14, P = n.s.
Adiponectin vs. T_3	r = –0.05, P = n.s.
Adiponectin vs. T_4	r = –0.06, P = n.s.
Adiponectin vs. ΔBw	r = –0.57, P = 0.03
Adiponectin vs. Food Intake	r = –0.07, P = n.s.

in plasma was also observed in our study, while no correlation was seen with body weight changes (Table 2).

Our study further showed that thyroid hormone treatment could result in a small but not statistically significant increase in ghrelin, a gastrointestinal hormone with stimulatory effects on food intake. This small increase though does not provide enough evidence to support a role for ghrelin in thyroxine-induced stimulation of food intake. It seems likely that ghrelin may have a compensatory role under the situation of reduced body mass, such as in hypothyroid animals. In fact, a marked increase in ghrelin levels was found in the hypothyroid rats (Table 1), and ghrelin levels were inversely correlated to body weight changes (Table 2), as has been previously shown [12]. However, this correlation was weak, and no correlation was seen with food intake.

Changes in adiponectin levels in plasma were also observed in relation to thyroid hormone changes. Adiponectin was not significantly altered in the plasma of the hyperthyroid animals despite the induced reduction in body weight gain by thyroid hormone (Table 1). It is now recognized that adiponectin has various effects on metabolism, which seem to be similar to those of thyroid hormone; they both induce mitochondrial biogenesis and increase energy expenditure [3, 12]. Thus, the fact that adiponectin was not changed by thyroxine administration probably implies that adiponectin may not be of physiological relevance in thyroxine-induced changes in energy expenditure. Interestingly, in our hypothyroid model, adiponectin was markedly increased. This could probably be considered as an adaptive response to reduced body weight associated with thyroid hormone changes (Table 1). In fact, adiponectin concentrations displayed an inverse relationship to body weight changes (Table 2), in agreement with previous observations [4]. In humans, till now, correlations of thyroid function and adiponectin levels are inconclusive, but, as already discussed, they do not necessarily apply to the laboratory animals [4, 13].

The present study has provided some new evidence regarding a potential role of adipokines, such as leptin and adiponectin, and the gastrointestinal hormone, ghrelin, in the regulation of energy homeostasis by thyroid hormone. To our knowledge, this is the first study, which has measured all three in an experimental model of hypo- and hyper-thyroidism. Thyroid hormone has recently attracted attention for its therapeutic potential in controlling body weight and reversing cardiac dysfunction of the failing heart [8, 14].

In conclusion, leptin seems mainly to be involved in the thyroid hormone action on energy homeostasis. Ghrelin and adiponectin may serve a compensatory physiological role in hypothyroidism

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