Calorific actions of leptin are additive to, but not dependent on, those of thyroid hormones

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Wang, Jin-Lin, Narumol Chinookoswong, Songmei Yin, and Zhi-Qing Shi. Calorific actions of leptin are additive to, but not dependent on, those of thyroid hormones. Am J Physiol Endocrinol Metab 279: E1278–E1285, 2000.—We examined a possible mechanistic interaction between leptin and thyroid hormones in rats with hypothyroidism induced by thyroidectomy (TX) and propylthiouracil administration. In study 1, the TX rats were treated by vehicle (V, n = 9) or by recombinant murine leptin (L, 0.3 mg·kg⁻¹·day⁻¹, n = 9) or were pair-fed (PF, n = 9) against L. In study 2, the TX rats were subdivided into three groups, namely, vehicle (T + V, n = 9), leptin (T + L, n = 10), and pair-feeding (T + PF, n = 9), similar to study 1 except for T₃ (T). Reduced food consumption and weight gain in the TX rats were reversed by T₃ replacement. Leptin suppressed food intake in the TX rats regardless of T₃ replacement. O₂ consumption (VO₂) and CO₂ production (VCO₂) were reduced in TX rats (P < 0.05 vs. normal) but were normalized by either T₃ or leptin treatment. T + L additively increased VO₂ and VCO₂ (P < 0.05 vs. TX, T₃, and L). The respiratory exchange ratio was unaltered in TX rats, with and without T₃, but was significantly reduced by L or T + L treatments. These results indicate that the metabolic actions of leptin are not dependent on a normal thyroid status and that the effects of leptin and T₃ on oxidative metabolism are additive.

The ob gene product leptin (19, 53) acts as a negative feedback signal in controlling metabolism and adiposity (4, 5, 30). Leptin deficiency in ob/ob mice is associated with a reduction in resting energy expenditure (20, 34). Systemic administration of leptin induces a significant reduction of body weight and food consumption and causes an increase in energy expenditure (9, 21, 34). 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. Recently, leptin was shown to prevent fasting-induced suppression of prothyrotropin-releasing hormone mRNA expression in the hypothalamic paraventricular nuclei (27). In our own studies, we have repeatedly documented elevated plasma thyroid hormone levels in rodents after prolonged leptin treatment (7, 49). Recent evidence shows that both leptin and thyroid hormone can stimulate uncoupling protein 3 mRNA expression in rodent muscle and brown adipose tissues (18). It is also well known that thyroid disorders in general are associated with changes in basal metabolic rate, oxygen consumption, appetite, and body weight (43). Thus the interactions between leptin and thyroid hormone were thought to be logical and may function as part of the leptin action mechanisms (25). However, the establishment of such interactions and mechanisms awaits more research evidence.

To examine our hypothesis that the effects of leptin on energy metabolism may be regulated independently of the thyrotropin-releasing hormone (TRH)/thyroid-stimulating hormone (TSH)/thyroid hormone axis, we have studied the effect of systemic administration of leptin on body weight, food consumption, and parameters of indirect calorimetry in thyroidectomized (TX) rats. The results obtained from the TX rats were compared with those obtained from normal control rats and also from TX rats with thyroid hormone replacement.

MATERIALS AND METHODS

Animal preparation. Male Sprague-Dawley rats (250–300 g) were obtained from Harlan Sprague Dawley (San Diego, CA). Animals were maintained at ambient temperature (22°C) and with 12:12-h light-dark cycles. Animals were fed a balanced rodent diet with a metabolizable energy of 2.98 kcal/g (Rodent diet no. 8640, Harlan Teklad, Madison, WI). Drinking water was allowed ad libitum. Hypothyroidism was induced by thyroidectomy performed by the vendor (Harlan Sprague Dawley). The TX rats were given 2% calcium acetate in drinking water for 10 days to prevent the hypocalcemia that might occur as a result of potential surgical impairment of the parathyroid glands. Propylthiouracil was given in drinking water (0.02%) for 4 wk to obviate residual, if any, thyroid hormone production (22). The hypothyroid status of these TX rats was verified by blood hormone levels (3,3’5’-
Experimental protocols. A total number of 55 TX rats were randomized into the following two studies to determine the effects of leptin in hypothyroid rats with (TX+T\textsubscript{3}) or without (TX) thyroid hormone replacement, respectively. The TX study was subdivided into three groups: 1) leptin treated (L, n = 9); 2) PBS vehicle treated (V, n = 9); and 3) vehicle treated and pair-fed, with a daily food ration matching the average daily consumption by the T+L group (T+PF, n = 9). The TX+T\textsubscript{3} study was performed in TX rats with T\textsubscript{3} replacement that were randomly divided into three subgroups similar to those in the TX study. The three subgroups in the TX+T\textsubscript{3} study were named, accordingly, as T+L (n = 10), T+V (n = 9), and T+PF (n = 8). Recombinant murine leptin (r-metMuleptin; Amgen, Thousand Oaks, CA) was administered (0.3 mg·kg\textsuperscript{-1}·day\textsuperscript{-1}) subcutaneously via implanted Alzet osmotic pumps (model 2ML4, ALZA, Palo Alto, CA). T\textsubscript{3} (Sigma, St. Louis, MO) was dissolved in PBS solution with 0.05% albumin (Sigma) and was administered subcutaneously via Alzet osmotic pumps (model 2004) at the rate of 5 μg·kg\textsuperscript{-1}·day\textsuperscript{-1}. This relatively low dose reportedly restored normal plasma T\textsubscript{3} levels and suppressed TSH in the hypothyroid rat (13). All animals were treated (leptin, T\textsubscript{3}, vehicle, and PF) for 3 wk. An additional group of eight healthy intact rats, matched for sex, age, and body weight, were studied in parallel with all of the other groups and served as the normal control group. All animals were monitored daily for changes in food consumption and body weight. The animal protocols were preapproved by the Laboratory Animal Research Committee of Amgen.

Determination of metabolic rate. The resting oxygen consumption (V\textsubscript{O\textsubscript{2}}), carbon dioxide production (V\textsubscript{CO\textsubscript{2}}), and respiratory exchange ratio (RER) were determined by use of an Oxymax indirect calorimetry system (Columbus Instruments). The effects of leptin on these parameters were compared with those of the normal controls and with the TX rats with and without T\textsubscript{3} replacement. After a 4- to 5-h fast and under nonstressful conditions, the rats were individually placed into indirect calorimetric chambers with 1-h adaptation time before the measurements. The measurements of flow, differential gas fractions (O\textsubscript{2} and CO\textsubscript{2}) across the chambers, were used to compute metabolic parameters. At the end of the experiments, all animals were killed under isoflurane anesthesia. Blood samples were collected via cardiac puncture for determination of plasma hormones and metabolites.

Determination of plasma hormones and metabolites. Baseline and terminal blood samples were obtained through tail artery of the rats under light isoflurane anesthesia. Blood was collected into heparinized (10 U/ml) polypropylene tubes and centrifuged immediately, and the plasma fractions were stored at -80°C until assayed. The biochemical parameters triglyceride, cholesterol, corticosterone, β-hydroxybutyrate (β-OHB), free fatty acids (FFA), lactate, and glycerol were measured spectrophotometrically on a Hitachi 717 Clinical Chemistry Autoanalyzer (Boehringer Mannheim, Indianapolis, IN). Plasma insulin concentration was measured with a rat insulin radioimmunoassay kit from Linco Research (St. Charles, MO) (31). Plasma leptin concentration was determined by an enzyme-linked immunooassay (51). The thyroid hormones (T\textsubscript{3} and T\textsubscript{4}) and TSH were determined in frozen plasma samples by Anlytics (Gaithersburg, MD) with radioimmunoassay methods (44).

Data analysis. All data are reported as means ± SE. Because of the large number of groups and various back-ground conditions, two statistical approaches were used to analyze the data. First, unpaired t-tests were used to compare the values between the two pairs: 1) N vs. V and 2) N vs. T+V. These comparisons identify the baseline differences of the hypothyroid rat model, with and without thyroid hormone replacement, compared with the euthyroid state. Second, two sets of ANOVA and Bonferroni-Dunn multicomparison tests were used for the TX cohort (including V, L, and PF groups) and for the TX+T\textsubscript{3} cohort (including T+V, T+L, and T+PF groups), respectively. An additional unpaired t-test was used to compare the T+L combined treatment with L alone to identify the impact of thyroid hormone replacement above leptin treatment. Statistical significance is defined as α < 5%. Calculations were done using the StatView program (SAS Institute, Cary, NC).

RESULTS

Effects of leptin on body weight and food consumption in hypothyroid rats. In contrast with the normal control rats that gained an average of 2 g/day, untreated TX rats (V) had no change in their mean body weight at the termination of the 3-wk experiment compared with the initial body weight (designated as 0, Fig. 1). Leptin treatment in TX rats resulted in a significant reduction in body weight compared with that of V (Δ = -1.81 g/day; P < 0.05, L vs. V; Fig. 1). This reduction was 50% greater than that of the PF rats (P < 0.01, PF vs. L). T\textsubscript{3} replacement in TX rats (T+V) caused a significant increase in body weight (Δ = 3.43 ± 0.20 g/day). However, such T\textsubscript{3}-induced increment in body weight is attenuated by two-thirds by either leptin treatment or restricted feeding (T+L, T+PF, both P < 0.05, vs. T+V). The mean daily energy intake was reduced in untreated TX rats (V) by 37% compared with normal control animals (146.1 ± 2.4 kcal·kg\textsuperscript{-0.75}·day\textsuperscript{-1}, P < 0.05, vs. V). Leptin treatment (L) and its corresponding pair-feeding (PF) further attenuated energy consumption by 20–25% from that of V (both P < 0.05). The reduced energy intake in TX rats (V) was significantly reversed by T\textsubscript{3} replacement with a 25% increment (T+V vs. V, P < 0.05). The leptin (T+L) and the corresponding pair-feeding control (T+PF) groups consumed only 80% of the daily energy consumption by the T+V group (both P < 0.05 vs. T+V).

Effects of leptin on V\textsubscript{O\textsubscript{2}} and V\textsubscript{CO\textsubscript{2}} in the hypothyroid rats. The resting V\textsubscript{O\textsubscript{2}} and V\textsubscript{CO\textsubscript{2}} were reduced by 11.4% (both P < 0.05, vs. V) alone for 1 and 3 wk (Table 1). T\textsubscript{3} replacement (T+V) alone for 1 and 3 wk normalized V\textsubscript{O\textsubscript{2}} (both P < 0.05 vs. V). Interestingly, leptin treatment alone (L) for 1 and 3 wk was able to closely reproduce the effects of T\textsubscript{3} in normalizing V\textsubscript{O\textsubscript{2}} [both P = nonsignificant (NS) vs. V+T; and P < 0.05 vs. V], whereas the corresponding pair-feeding had no effect (P = NS, PF vs. V, for both 1 and 3 wk). More intriguingly, combined treatment of leptin and T\textsubscript{3} (T+L) resulted in an apparently additive effect in stimulating V\textsubscript{O\textsubscript{2}} (15.34 ± 0.38 ml·kg\textsuperscript{-1}·min\textsuperscript{-1} at 3 wk, P < 0.05 vs. L and T+V), The changes in V\textsubscript{CO\textsubscript{2}} induced by 3 wk of T\textsubscript{3} treatment (T+V) are qualitatively similar to the change from leptin (L). Again, the combined treat-
were food-restricted (T+PF), the $V_O_2$ and $V_CO_2$ were initially normalized (at 1 wk). However, the prolonged undernourishment (3 wk) results in a further increase in $V_O_2$ but a decline in $V_CO_2$.

**Effect of leptin on RER in TX rats.** With both reduced $V_O_2$ and $V_CO_2$, the RER in untreated TX rats was not significantly different from that of normal control rats at both 1 and 3 wk of the study (Fig. 2). RER with leptin treatment alone (L) was significantly reduced to $0.803 \pm 0.01$ at 1 wk ($P < 0.05$ vs. V) and regained slightly at 3 wk ($0.844 \pm 0.010$). Reduced feeding in hypothyroid rats (PF) resulted in $9.0\%$ lower RER values than those in L ($P < 0.001$). $T_3$ replacement ($T+V$) normalized RER (at 1 and 3 wk, $P = NS$ vs. respective N values). Leptin treatment combined with $T_3$ replacement ($T+L$) displayed a biphasic change in RER that was significantly reduced at 1 wk but became elevated to the same level as the normal control (N) and $T_3$-treated (T+V) groups at 3 wk. The RER values were initially elevated ($0.913 \pm 0.006$, 1 wk) but later declined ($0.764 \pm 0.025$, 3 wk) in the $T_3$-replaced food-restricted (T+PF) rats, in contrast to those in the T+L group.

**Effects of leptin on hormones and metabolites in TX rats.** As shown in Table 2, markedly diminished total free $T_3$ levels confirm the hypothyroid status in all of the TX rats (V, L, and PF), which was totally normalized or substantially improved in the TX+T3 rats (T+V, T+L, and T+PF). The extremely diminished total and free $T_3$ levels in the TX animals were not affected by $T_3$ replacement. All hypothyroid rats had significantly elevated TSH levels (5- to 6-fold increments in V, L, and PF) from the level of N ($2.2 \pm 0.35$ ng/ml). The increment of TSH was attenuated by $T_3$ replacement for 3 wk (1.6- to 4-fold increments in T+V, T+L, and T+PF), with T+PF showing the least increment. These data support the validity of our TX model and the replacement therapy. Plasma leptin concentrations were not affected by hypothyroidism (V vs. N, $P = NS$) but were significantly decreased ($P < 0.05$) by reduced feeding with PF ($P < 0.05$, vs. N and V) and T+PF ($P < 0.05$ vs. N and T+V). Leptin treatment significantly raised plasma leptin concentrations in both L and T+L groups, but the magnitude of increment was far greater in L than in T+L. Corticosterone levels were 30% lower in the hypothyroid rats ($9.92 \pm 0.27$ vs. N $12.50 \pm 0.20$) with both reduced feeding and hypothyroidism (PF) associated with markedly reduced $V_CO_2$ ($9.37-9.49$ ml$\cdot$kg$^{-1}$$\cdot$min$^{-1}$) from V, whereas the $V_O_2$ was not different from that of V, apparently in line with the hypothyroidism and reduced feeding conditions. When $T_3$-treated animals were initially normalized (at 1 wk).

**Table 1. Effects of leptin on $V_O_2$ and $V_CO_2$ in TX rats with or without thyroid hormone replacement**

<table>
<thead>
<tr>
<th>Treatment, wk</th>
<th>Normal</th>
<th>V</th>
<th>L</th>
<th>PF</th>
<th>T+V</th>
<th>T+L</th>
<th>T+PF</th>
</tr>
</thead>
<tbody>
<tr>
<td>n = 8</td>
<td>V</td>
<td>L</td>
<td>PF</td>
<td>T+V</td>
<td>T+L</td>
<td>T+PF</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>13.36±0.62</td>
<td>12.98±0.35</td>
<td>13.83±0.33</td>
<td>12.95±0.35</td>
<td>14.69±0.39</td>
<td>14.57±0.36</td>
<td>13.28±0.24</td>
</tr>
<tr>
<td>3</td>
<td>13.99±0.66</td>
<td>12.40±0.35</td>
<td>13.74±0.46</td>
<td>12.32±0.18</td>
<td>13.54±0.39</td>
<td>13.54±0.38</td>
<td>14.56±0.39</td>
</tr>
<tr>
<td>VCO2, ml$\cdot$kg$^{-1}$$\cdot$min$^{-1}$</td>
<td>11.66±0.56</td>
<td>11.03±0.38</td>
<td>11.13±0.27</td>
<td>9.49±0.26</td>
<td>12.50±0.29</td>
<td>11.32±0.34</td>
<td>12.03±0.21</td>
</tr>
<tr>
<td>3</td>
<td>11.83±0.48</td>
<td>10.89±0.34</td>
<td>11.56±0.35</td>
<td>9.37±0.16</td>
<td>11.29±0.41</td>
<td>12.72±0.35</td>
<td>11.10±0.44</td>
</tr>
</tbody>
</table>

Effects of leptin on resting indirect calorimetry in thyroidectomized (TX) rats after 1 or 3 wk of leptin and/or 3,3’5’-triiodo-L-thyronine ($T_3$) treatment. $V_O_2$, oxygen consumption; $V_CO_2$, carbon dioxide production. Leptin (0.3 mg$\cdot$kg$^{-1}$$\cdot$day$^{-1}$) was infused subcutaneously. TX control animals received vehicle (V) infusion alone or plus pair-feeding (PF) treatment for 3 wk. $T_3$ (5 μg$\cdot$kg$^{-1}$$\cdot$day$^{-1}$) replacement therapy was given in 3 groups (T+V, T+L, and T+PF). Intact rats served as a normal control group. Values represent means ± SE. *P < 0.05, V or T + V vs. Normal; †P < 0.05, L vs. V (or T+L vs. T+V); ‡P < 0.05, L vs. PF (or T+L vs. T+PF); §P < 0.05 L vs. T + L.
erol was moderately lowered (12%) by TX (L, T) and reduced feeding (PF and T) half (P < 0.05, V vs. N) and were further attenuated (P < 0.05) in the hypothyroid state than in the normal state (both P < 0.01). The levels of β-OHB are often used as an indicator of fatty acid oxidation. No apparent changes were observed in hypothyroid rats except for the pair-fed groups, PF and T+PF, in which the levels of β-OHB were found substantially elevated (both P < 0.01, vs. N, V, L, T+V, and T+L groups).

DISCUSSION

The present study demonstrates for the first time that leptin is capable of stimulating energy metabolism in the hypothyroid state in the rat, evidenced by correction of reduced Vo2 and VCO2. The effects of leptin are comparable to those of thyroid hormone replacement therapy. Notably, after just 1 wk of leptin treatment, Vo2 was significantly elevated, whereas VCO2 remained as low as that of untreated hypothyroid rats. This suggests an overall state of increased energy metabolism, with a greater contribution to calorigenesis by fat oxidation in the early stage of leptin treatment (18, 20, 21). After 3 wk of leptin treatment, VCO2 was also significantly increased and brought back to the normal levels. This, together with a normalized Vo2 value, led to a normal RER. These data indicate that the metabolic rate stimulated by leptin was initially (at 1 wk) overweighted with fat oxidation but that later (after 3 wk) was transitioned toward the use of more balanced energy substrates (fat, carbohydrate, and protein). Our data strongly suggest that the effects of leptin in modulating energy metabolism are not mediated by secondary thyroid messages and are not dependent on a normal thyroid status. We also demonstrate that a prolonged combined treatment using leptin and T3 in hypothyroid rats results in an additive enhancement in energy consumption, reflected by substantially increased total Vo2. A relatively low but effective dose of T3 replacement (13) was chosen in this study to avoid artificial hyperthyroidism, which could obscure the biological actions of leptin. Such slight underreplacement may affect a number of parameters, including an insufficient increase in energy intake (seen in the T+V group).

Hypothyroidism is an insufficiency in thyroid hormone production and/or function, with elevated TSH secretion resulting from a lack of negative-feedback stimulation to the hypothalamus and pituitary. Thyroid insufficiency leads to a generalized slowing of calorigenesis (8, 39). The untreated hypothyroid rats (V)
and thus a regain of the retarded growth. This regain placed while energy intake was increased. In the leptin treatment in both normal (49) and diabetic rats demonstrated significant increases in plasma T4 levels after mRNA expression in the hypothalamic paraventricular venting fasting-induced suppression of pro-TRH can also impact on thyroid hormone secretion by pre-modulation of thyroid activities (23, 40, 46, 50). Leptin involved in the leptin action mechanism (18, 25, 27, 33). has been postulated that the TRH-TSH-T4 axis may be involved in the leptin action mechanism (18). These effects appear to coincide with the physiological role of the thyroid hormones (18, 26). Thus, it has been postulated that the TRH-TSH-T3 axis may be involved in the leptin action mechanism (18, 25, 27, 33).

Leptin has been found to influence thyroid hormone secretion or circulating levels (1, 27, 33). Systemic or central administration of leptin reportedly induces a myriad of neuroendocrine responses. Among all the neuroendocrine responses, neuropeptide Y and glucocorticoid hormones are thought to be implicated in modulation of thyroid activities (23, 40, 46, 50). Leptin can also impact on thyroid hormone secretion by preventing fasting-induced suppression of pro-TRH mRNA expression in the hypothalamic paraventricular nuclei (1, 27). In our previous studies, we have demonstrated significant increases in plasma T3 levels after leptin treatment in both normal (49) and diabetic rats (7). These observations seem to imply a significant involvement of the thyroid gland in the leptin action mechanism.

If thyroid hormones indeed constitute a major component of the action mechanism of leptin, then removal of thyroid hormones would logically obliterate or weaken leptin's metabolic actions. However, leptin treatment alone, in the current study in the hypothyroid rats, was nearly as potent as in the intact animals in reducing food intake and body weight and in stimulating oxidative and calorigenic metabolism. On the other hand, T3 replacement alone in TX rats was as effective as exogenous leptin treatment in enhancing oxygen consumption and CO2 production. Thus leptin and thyroid hormones appear to run in parallel and act independently of each other to achieve comparable calorigenic effects. These two hormones appear to invoke distinct mechanisms and yet act in concert and additively to modulate energy metabolism. The mechanistic distinction between leptin and thyroid hormones can also be appreciated in the changes of energy intake and body weight. The lower-than-normal daily energy intake in hypothyroid (V) rats was further reduced by leptin (L, −25%), whereas it was increased by 33% with T3 replacement (T+V). Furthermore, the TX rats suffered an initial growth stunt due to hypothyroidism. After treatment with T3, they appeared to have recaptured part of the delayed growth. In contrast, leptin either caused a net weight loss in TX rats or attenuated T3-induced weight gain in T+L rats. These differences, taking place in the face of comparable calorimetric enhancement by leptin or T3, argue against a mechanistic dependence of the leptin action on thyroid hormones. Although leptin may induce a measurable increment in thyroid hormone release, the absence of thyroid hormones does not hamper the metabolic actions of leptin. Thus the thyroid gland does not

### Table 2. Effect of leptin on plasma hormones and metabolites in TX rats with or without thyroid hormone replacement

<table>
<thead>
<tr>
<th>Hormones</th>
<th>Normal</th>
<th>TX (n = 9)</th>
<th>TX + L (n = 10)</th>
<th>TX + PF (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total T3, ng/dl</td>
<td>58.31</td>
<td>17.64</td>
<td>15.72</td>
<td>19.49</td>
</tr>
<tr>
<td>Free T3, ng/dl</td>
<td>0.56</td>
<td>0.02</td>
<td>0.03</td>
<td>0.02</td>
</tr>
<tr>
<td>Total T4, µg/dl</td>
<td>4.71</td>
<td>0.03</td>
<td>0.01</td>
<td>0.06</td>
</tr>
<tr>
<td>Free T4, ng/dl</td>
<td>1.31</td>
<td>0.02</td>
<td>0.05</td>
<td>0.02</td>
</tr>
<tr>
<td>TSH, ng/ml</td>
<td>2.2</td>
<td>27.15</td>
<td>27.91</td>
<td>30.98</td>
</tr>
<tr>
<td>Leptin, ng/ml</td>
<td>1.49</td>
<td>1.56</td>
<td>43.71</td>
<td>0.80</td>
</tr>
<tr>
<td>Insulin, ng/dl</td>
<td>1.36</td>
<td>0.40</td>
<td>0.17</td>
<td>0.10</td>
</tr>
<tr>
<td>Corticosterone, µg/dl</td>
<td>14.55</td>
<td>9.92</td>
<td>11.31</td>
<td>15.42</td>
</tr>
</tbody>
</table>

Plasma hormones and metabolites were measured after 3 wk of leptin and/or thyroid hormone treatment as described in MATERIALS AND METHODS. T3, thyrone; TSH, thyroid-stimulating hormone; β-OHB, β-hydroxybutyrate; UD, undetectable. Values represent means ± SE.

\*P < 0.05, V or T + V Normal; \#P < 0.05, L vs. V (or T + L vs. T + V); \%P < 0.05, L vs. PF (or T + L vs. T + PF); \$P < 0.05 L vs. T + L.
constitute an essential link of the leptin action pathway.

The values of the RER reflect the sources of oxidative energy substrates. An RER value close to 1, or to 0.7, indicates a predominant oxidation of carbohydrates, or fat, respectively (24, 28). Among the hypothyroid groups with T3 replacement (TX+T3) for 1 wk, the RER values of the leptin-treated group (T+L) were significantly lower than the level of the vehicle control (T+V) and the pair-fed (T+PF) groups. This indicates that the animals treated with both leptin and T3 utilized more fat as the oxidative fuel. This effect was nevertheless contributed primarily by leptin, which by itself was able to reduce RER while increasing VO2. On the other hand, T3 replacement alone had no effect on RER values, because both VO2 and VCO2 increased in proportion. It may be deduced that leptin plays a more important role in metabolizing fat than do the thyroid hormones. The discrepancies in the RER, VO2, and VCO2 values and in their interrelationship invoked by leptin vs. thyroid hormone further support our thesis that the two hormones are mechanistically distinct.

In light of the similar and yet mutually independent metabolic effects of leptin and thyroid hormones, we hypothesize that a common downstream effector site may exist as a converging point for both hormones. One such possible downstream effector may be the sympathetic nervous system (9). Leptin has been shown to stimulate thermogenic metabolism (39), probably involving activated uncoupling proteins (18, 29). Thyroid hormone can also potentiate the sympathetic neural activities (36, 37) and enhance uncoupling protein expression (15). An alternative downstream site could be the growth hormone (GH), which can be stimulated by either leptin (6) or thyroid hormones (16). GH is affected by nutritional states and has well established effects of stimulating oxidative metabolism (VO2) (32, 48). Although these two potential factors are discussed, the actual mechanisms are still poorly understood and await further and more direct evidence.

Leptin has been shown to raise circulating thyroid hormone levels in both normal (49) and diabetic rats (7), but the effect of altered thyroid status on leptin levels is rather controversial. Circulating leptin levels were reportedly elevated (35), decreased (12, 47), or unchanged (10, 45) in hypothyroidism. Body mass index and gender appear to remain the major correlates of circulating leptin concentrations in each of the altered thyroid states, as in the general population (10, 45). In the present study, leptin levels were not significantly affected by the hypothyroid state (P = NS, N vs. V). Thyroid hormones at pharmacological levels stimulate lipogenesis and OB gene expression in vitro (14, 52), whereas the slightly underreplaced T3 in these hypothyroid rats (TX+T3) did not significantly increase leptin levels. The lower leptin levels in the food-restricted groups (PF and T+PF) are likely the consequence of a net catabolic state and a negative weight gain. It is interesting that the leptin concentrations in leptin-treated TX rats (L) were 2.6-fold higher than those in rats with combined leptin and T3 treatment (T+L) despite the same rates of leptin delivery. Leptin is known to be almost totally cleared by the kidneys (11), and hypothyroidism is associated with a significantly impaired renal clearance for creatinine (38). Thus the correction of hypothyroidism by T3 replacement may presumably enhance renal clearance of leptin and attenuate the rise in leptin levels induced by exogenous leptin in the T+L group.

The reduction in plasma insulin levels by leptin is consistent with our previous observations in both intact (49) and diabetic rats (7) and relates to decreases in energy intake, body weight, and blood glucose levels. Reduced levels of corticosterone in the TX rats are likely due to an attenuated tone of the hypothalamic/pituitary/adrenal axis in hypothyroidism and appeared to be corrected by T3 replacement. Leptin treatment (L and T+L) attenuated the decrease of corticosterone in both TX and T+T3 rats and also minimized the fasting-induced rise (as seen with PF and T+PF) in circulating corticosterone, in agreement with the other reports (1, 3).

The significant body weight loss in leptin-treated rats has been attributed mainly to the loss of visceral fat (2). This may also be the case in the hypothyroid rats, although body composition was not determined. The falling values of triglycerides in leptin-treated rats (L and T+L) may be related to reduced production (41), increased oxidative metabolism in the adipose tissue (49), and a decrease in visceral fat mass (2). Elevated cholesterol levels in hypothyroid rats were not corrected by T3 treatment for 3 wk in our experimental setting. The significant decrease in plasma triglyceride is in part due to a decrease in triglyceride synthesis (7, 41) and in part due presumably to an accelerated fat decomposition and oxidation (49). The decrease in circulating FFA levels in TX rats presumably resulted from a diminished rate of lipolysis, which appears to be normalized by leptin but not by T3 replacement. Although the L and T+L rats consumed the same amount of food as their corresponding pair-fed groups (PF and T+PF groups), respectively, leptin treatment appeared to be able to normalize the β-OHB levels regardless of whether hypothyroidism was corrected with T3. This may be related to a counterbalance of both increased ketone body production and utilization, because leptin is known to stimulate ketosis in normal rats (49). The PF and T+PF groups displayed a unique pattern of hormones (such as cortisol, leptin, insulin, and T3) and metabolites (such as cholesterol, triglyceride, glycerol, β-OHB, and FFA) that are distinct from those of leptin treatment, although their food intake was equivalent. These differences are in agreement with the current consensus that the metabolic effects of leptin are largely independent of reduced food intake.

In summary, our study demonstrates for the first time that the effects of leptin in reducing food intake and body weight and in stimulating energy metabolism are not dependent on the presence of the thyroid hormones. The thyroid gland does not constitute an integral component of the leptin action mechanism. However, leptin and thyroid hormones may share some common downstream action sites and can thus act
additively, although independently, to enhance calori-
genic metabolism.

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