Regulation of Leptin by Thyroid Hormone in Humans: Studies In Vivo and In Vitro

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The influence of thyroid hormones on human adipose tissue leptin production and leptin gene expression was investigated in vitro and in vivo. Twelve women received 60 μg triiodothyronine (T_3) per day for 7 days, which increased total T_3 by 195% (1.78 ± 0.07 to 5.25 ± 0.39 mU/L, $P < .001$), significantly decreased thyrotropin ([TSH] 1.57 ± 0.40 to 0.03 ± 0.01 mU/L, $P < .01$), and increased energy expenditure (1,602 ± 32 to 1,754 ± 34 kcal/24 h, $P < .05$). However, serum leptin did not change (9.36 ± 1.6 *v* 8.90 ± 1.3 μg/L, nonsignificant). Human subcutaneous adipose tissue biopsies from eight healthy women were incubated in vitro as small fragments with T_3 in concentrations from 1 to 50 nmol/L. Leptin production was inhibited dose-dependently. After 24 hours of incubation, a T_3 concentration of 50 nmol/L reduced basal leptin production by 42% ($P < .05$) and the stimulated leptin production (dexamethasone 10 nmol/L) by 52% ($P < .05$). Leptin mRNA expression was measured by a semiquantitative multiplex reverse transcriptase–polymerase chain reaction (RT-PCR) method. Fifty nanomolars T_3 decreased basal leptin mRNA expression by 47% compared with controls ($P < .001$), and the stimulated leptin mRNA expression was reduced to a similar degree (53%). In conclusion, in human adipose tissue, T_3 (>20 nmol/L) inhibited leptin production and leptin gene expression in vitro, whereas an elevation of T_3 corresponding to a moderate thyrotoxic state (T_3 5.25 ± 0.39 nmol/L) was without any impact on serum leptin levels in vivo.

PLASMA LEPTIN is highly correlated with the body mass index (BMI) and other indices of adiposity.[1] In agreement with this, plasma leptin is increased in obesity and decreased in anorexia nervosa,[2] reflecting the changes in adipose tissue mass. Leptin is mainly supposed to signal the amount of adipose tissue to the brain, but a number of hormones regulate the leptin level and may cause it to be out of proportion to the amount of adipose tissue. Leptin levels are stimulated by insulin, dexamethasone, and some cytokines and inhibited by β-adrenergic receptor stimulation and possibly testosterone.[3] In normal mice and *ob/ob* mice, which lack endogenous leptin production, leptin treatment decreases body fat, increases energy expenditure, and depresses the appetite, leading to a reduction in body weight.[4]

Thyroid hormones have marked metabolic effects such as increased thermogenesis via uncoupling of oxidative phosphorylation through uncoupling proteins,[5] an increased basal metabolic rate, and increased mobilization of triglyceride from the adipose tissue.[6] Moreover, thyroid dysfunctions are often associated with changes in body weight and appetite.

The common effect of leptin and thyroid hormones on energy expenditure, adipose tissue mass, and appetite raises the question of an interaction between leptin and the thyroid hormone axis. In accordance with this, investigations have shown that leptin prevents the fasting-induced decrement in pro-thyrotropin (TSH) mRNA in the hypothalamic paraventricular nucleus.[7] The effects of the thyroid status on leptin levels have also been investigated, but the data are contradictory. In humans, decreased serum leptin levels in hypothyroid patients have been reported in comparison to controls matched for the BMI, age, and sex, and leptin was partially normalized after replacement therapy.[8] However, others have found that neither hyperthyroid nor hypothyroid states were associated with alterations in serum leptin concentrations.[9] On the contrary, two recent studies in which body composition was estimated by bioelectrical impedance or dual-energy x-ray absorptiometry have reported relatively low levels of leptin in hyperthyroidism[10,11] and an increase in leptin after treatment of the disease.[11] The decrease in leptin was found despite a relative increase in body fat, and is consistent with a negative effect of thyroid hormone on leptin levels as found in some animal studies.[12,13]

To provide further insight into the relationship between thyroid hormones and leptin in humans, we measured plasma leptin concentrations during short-term triiodothyronine (T_3) treatment of healthy premenopausal women. Moreover, the direct effect of T_3 on human subcutaneous adipose tissue in vitro was investigated.

SUBJECTS AND METHODS

Subjects

Twelve healthy premenopausal women were studied. Their body weight was stable for at least 2 months as indicated by their history, and none of the women were on contraceptive medication. The women were included in the study after provision of informed consent and in accordance with the Helsinki Declaration. The study was approved by the local ethics committee. Every morning for 7 days, the women were treated with 60 μg T_3, which produces a hyperthyroid state within 7 days.[14] Blood samples were drawn after a 12-hour fast at day 0, at day 8 (24 hours after the last T_3 dose), and at day 14 (7 days after T_3 treatment was stopped).

Anthropometric Measurements

Height was measured to the nearest 0.5 cm and body weight to the nearest 0.1 kg. The BMI (weight in kilograms divided by height in meters squared) was calculated. The heart rate was measured from an electrocardiogram.

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Blood Parameters

The leptin level was measured in serum samples by a radioimmunoassay (RIA) method (Linco Research, St. Charles, MO). The range of the standard curve in this assay is 0.5 to 100 ng/mL. The intraassay coefficient of variation was 3.7%. No cross-reaction is observed between the antibody in the assay and human insulin, proinsulin, glucagon, pancreatic polypeptide, or somatostatin. Thyroid hormones, TSH, and bone parameters were measured as previously described.[14]

Resting Energy Expenditure

Resting energy expenditure (REE) was measured before and after stimulation with thyroid hormones in seven patients. REE was assessed by indirect calorimetry for 30 minutes in a room with a temperature between 20° and 24°C. The subjects fasted from midnight, did not perform any physical activity on the day before the measurements, and were brought to the hospital in the morning. The subjects rested 30 minutes before the measurements, and the initial 10 minutes of calorimetry was used for acclimatization. A computerized open-circuit system measured gas exchange across a 25-L canopy (Deltatrac; Datex Instrumentarium, Helsinki, Finland). The monitor determines carbon dioxide production and oxygen consumption by multiplying dry airflow through the canopy with alterations in gas concentrations over the canopy. Net glucose and lipid oxidation rates were calculated from these measurements, and protein oxidation rates from 24-hour urinary excretion of urea.

Incubation of Adipose Tissue Fragments

Subcutaneous adipose tissue was obtained by liposuction from the abdominal region in eight healthy premenopausal women. Briefly, the skin was anesthetized using 10 mL lidocaine (10 mg/mL). A small incision was made and isotonic NaCl was injected into the adipose tissue. A 4-mm liposuction cannula was inserted and the vacuum was applied. A volume of 10 to 20 mL tissue was removed using sterile technique and then placed in medium 199, and all subsequent procedures were performed under a laminar-airflow hood. The tissue was minced into fragments of less than 10 mg each. All samples were washed free of blood clots and free lipid and placed in organ culture as previously described.[15,16] In brief, 500 mg adipose tissue fragments floated freely in 16 mL serum-free medium 199 without phenol red in 50-mL plastic tubes. The cultures were placed in a humidified incubator and maintained at 37°C in an atmosphere of 5% CO_2. Medium 199 was supplemented with 25 mmol/L HEPES, 5% bovine albumin, and 1 nmol/L insulin (Novo Nordisk, Lyngby, Denmark). For each tested variable in either untreated or treated samples, the adipose tissue was incubated in duplicate and the medium was collected after 24 hours for leptin analyses. Leptin production was measured in the basal condition without hormone addition and after stimulation with T_3 at different concentrations (1, 10, 20, and 50 nmol/L). The hormones were added at the initiation of the culture. Because of the pronounced and reproducible responses to dexamethasone, all incubations were performed with dexamethasone as a control to ensure the responsiveness of the cells. In addition, to investigate the effect of T_3 in a situation with stimulation of leptin secretion 50 nmol/L, T_3 was also incubated together with 10 nmol/L dexamethasone. The adipose tissue was immediately frozen in liquid nitrogen and kept at −80°C until RNA isolation. The culture medium was kept at −20°C until leptin analysis. Leptin was measured in culture medium using a human super-sensitive RIA (range, 0.05 to 10 ng/mL; Linco Research). The intraassay coefficient of variation was 5.8%.

Leptin mRNA Quantitation

RNA was isolated using the Trizol reagent (GIBCO-BRL, Gaithersburg, MD). Reverse transcription and amplification were performed with 25 ng total mRNA using Amplitaq Gold DNA polymerase and hexamer primers as described by the manufacturer (GeneAmp PCR kit; Perkin Elmer Cetus, Norwalk CT). The leptin primers used in the polymerase chain reaction (PCR) spanned a cDNA product of 352 base pairs. The sense leptin primer corresponds to nucleotides 85 to 107 and the antisense strand of nucleotides 417 to 437.[17] β-Actin mRNA was amplified as a housekeeping marker, and a semiquantitative multiplex PCR method, "primer-dropping,"[18] was used to monitor mRNA expression. Semiquantitative multiplex PCR estimates the relative amount of mRNA to a known housekeeping gene (β-actin) as an internal control of the sample variability. Initial experiments were performed for each set of primers to determine the cycle number for exponential amplification of cDNA (data not shown). The target mRNA (leptin) is present at much lower levels compared with β-actin. Therefore, if the target primers were run 11 PCR cycles before the β-actin primers were dropped (primer dropping), both sets were brought into the middle of the exponential range after an additional 22 cycles. A similar set-up was used for negative controls, but the reverse transcriptase (RT) was omitted and no PCR products were detected. PCR products were loaded on a 2% agarose gel stained with ethidium bromide and analyzed using the Bio-Rad (Richmond, CA) Gel Doc 1000 system for quantification. The coefficient of variation was 10.2%.

Statistical Analysis

Data are presented as the mean ± SEM. The SPSS statistical package (SPSS/PC+; SPSS, Chicago, IL) was used. The distribution of leptin was normalized by natural logarithmic transformation in the in vivo study. Student's paired *t* test or nonparametric tests (Mann-Whitney) were used for comparisons between group means. Simple linear regression analyses were used to relate variables. Covariance analysis was used to adjust leptin levels for the BMI. A P value less than .05 was considered significant (two-way significance).

RESULTS

Basal Characteristics

The age of the subjects was 49.0 ± 0.54 years (range, 45 to 51) and they were non-obese (BMI, 24.4 ± 0.92 kg/m^2). All subjects were premenopausal based on their medical history and follicle-stimulating hormone (FSH), luteinizing hormone (LH), and estradiol measurements. Baseline blood samples including thyroid hormones were normal (Table 1). The mean serum leptin was 9.4 ± 1.6 μg/L.

Effects of T_3

After 7 days of oral treatment with 60 μg T_3, a moderate thyrotoxic state was achieved. Mean serum total T_3 increased about threefold compared with prestimulation values ($P < .001$), and after 1 week without T_3, the mean T_3 returned to prestimulation values ($P = .21$; Table 1). Serum free T_3 was increased 3.4-fold at day 7 ($P < .001$) and returned to below prestimulation levels at day 14 ($P < .05$). Serum total thyroxine was decreased at day 8 by 26% ($P < .001$) and at day 14 by 30% ($P < .001$). TSH was reduced to 2% of the prestimulation level at day 7 ($P < .001$) and returned to the baseline level at day 14 ($P = .5$). Bone parameters confirmed the thyrotoxic state (Table 1). During the treatment period, the participants lost 1,000 ± 19 g (range, 100 to 1,900), or 1.5% of their body weight ($P < .001$). Seven days after cessation of T_3 treatment, the weight still had not returned to the baseline (−910 g; range, 80 to 2,600; $P < .003$). The heart rate, measured in the morning after a 30-minute rest period, increased from 72.3 ± 2.5 to

Table 1. Characteristics of the Subjects

Parameter	Day 0	Day 8	Day 15
Serum leptin (μg/L)	9.36 ± 1.58	8.9 ± 1.25	9.2 ± 1.47
Thyroid hormones			
s-TT_3 (nmol/L)	1.78 ± 0.07	5.25 ± 0.39†	1.69 ± 0.09
s-FT_3 (pmol/L)	4.66 ± 0.17	15.69 ± 1.21†	4.40 ± 0.11
s-TT_4 (nmol/L)	120.67 ± 7.66	87.58 ± 6.06†	83.42 ± 5.50*
s-FT_4 (pmol/L)	15.67 ± 1.09	10.58 ± 1.01†	9.08 ± 0.92†
s-TSH (mU/L)	1.57 ± 0.40	0.03 ± 0.01†	1.82 ± 0.45
Other measurements			
BMI (kg/m^2)	24.4 ± 0.9	24.0 ± 0.9†	24.1 ± 0.9*
REE (kcal/24 h)	1,602 ± 32	1,754 ± 34*	—
Lipid oxidation (g/24 h)	73.7 ± 5.3	85.3 ± 5.9	—
Heart rate (min^{-1})	72.3 ± 2.5	82.0 ± 3.2*	70.7 ± 2.7
Bone parameters			
s-Osteocalcin (μg/L)	15.4 ± 1.3	21.5 ± 1.7†	14.2 ± 1.2
u-Hydroxyproline (μmol/μmol creatinine)	150.8 ± 10.9	212.1 ± 15.4†	181.6 ± 15.4*

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Abbreviations: s-TT_3, serum total T_3; s-TT_4, serum total T_4; s-FT_3, serum free T_3; s-FT_4, serum free T_4.

Student's paired *t* test: *$P < .05$ *v* day 0; †$P < .001$ *v* day 0.

82.0 ± 3.2, or 13.9% ($P < .004$), and returned to normal after 1 week without treatment. REE increased from 1,602 ± 32 to 1,754 ± 34 kcal/24 h, or 9% ($P < .01$), and oxygen consumption increased from 234.4 ± 4.9 to 256.6 ± 4.7 mL/min ($P < .05$). T_3 treatment did not change net glucose oxidation significantly, but a strong tendency for an increase in the lipid oxidation rate was found ($P = .06$; Table 1).

Serum leptin was insignificantly decreased by 5.6%, from 9.4 ± 1.6 to 8.9 ± 1.3 μg/L ($P = .5$), after 7 days of thyroid hormone treatment. Leptin did not change from day 7 to day 14 (Fig 1). Normalization of serum leptin to the body weight or BMI did not change the results ($P = .76$). Leptin correlated highly positively with the BMI ($r = .70$, $P < .001$). The associations between leptin and free T_3, free thyroxine, TSH, or REE or between the delta values were investigated at the three different time points, but no associations were found. Finally, no correlation was found between leptin and FSH or leptin and LH (N = 12).

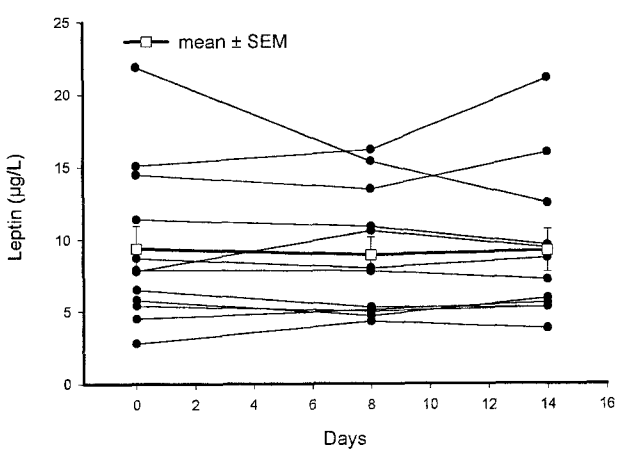

Fig 1. Effects of T_3 on serum leptin. Leptin is shown for each subject during the study. T_3 (60 μg/d) was administered from day 1 to day 7.

Effects of T_3 on Adipose Tissue In Vitro

Subcutaneous adipose tissue fragments responded to dexamethasone 10 nmol/L with a 2.5-fold increase in leptin secretion into the media upon 24 hours of stimulation ($P < .05$; Fig 2). Thyroid hormones were added at 1 to 50 nmol/L to the culture medium (containing 5% bovine serum albumin). Leptin secretion was dose-dependently inhibited by thyroid hormone, and the inhibition became significant at a T_3 dose above 20 nmol/L after 24 hours of incubation ($P < .05$). Basal leptin production from 500 mg adipose tissue was about 1.2 ng/mL for 24 hours, corresponding to 38.4 ng leptin per gram of adipose tissue. To determine if the effects of T_3 in the basal situation could be reproduced during stimulated conditions, dexamethasone was added to the medium. Our previous studies have shown that a dexamethasone concentration of 10 nmol/L produces submaximal stimulation of leptin secretion. We found that the stimulated leptin secretion was inhibited to a similar extent (53%) as the basal secretion after 24 hours of incubation with 50 nmol/L thyroid hormone ($P < .05$). Semiquantitative multiplex PCR revealed that the inhibitory effect of T_3 also was found on the transcriptional level. Fifty nanomolars of T_3 significantly decreased leptin expression by 47% compared with controls ($P < .001$, n = 8). When leptin was stimulated with dexamethasone, leptin expression was inhibited to a similar degree by T_3 ($P < .02$, n = 8; Fig 3).

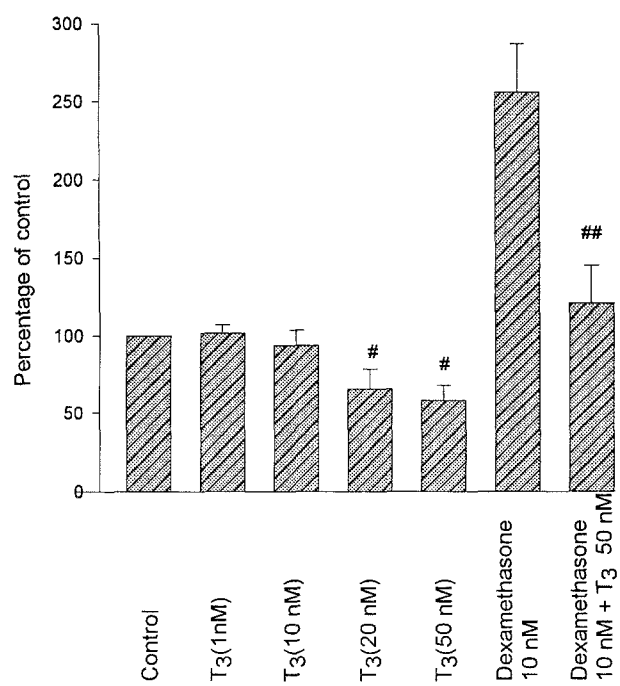

Fig 2. Effects of T_3 on human subcutaneous adipose tissue leptin production in vitro for 24 hours (n = 8). *$P < .05$ *v* control; **$P < .05$ *v* dexamethasone (10 nmol/L) (Mann-Whitney test).

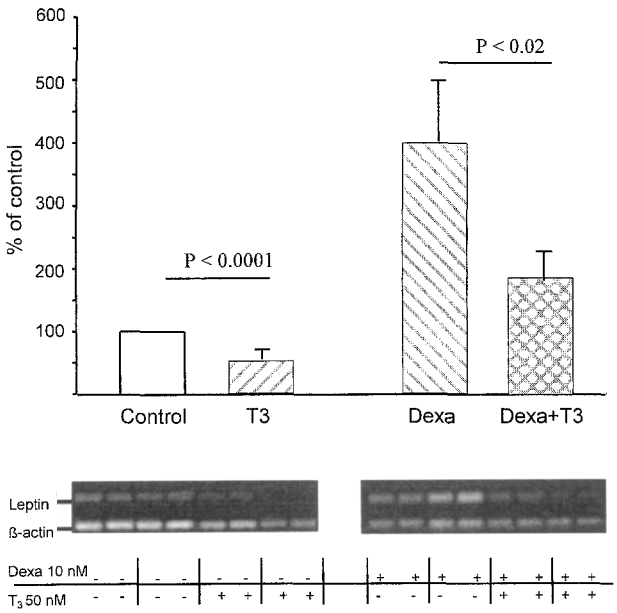

Fig 3. Effects of T_3 on human subcutaneous adipose tissue leptin gene mRNA expression in vitro. Incubations were performed for 24 hours in the presence or absence of T_3 (50 nmol/L) and dexamethasone (10 nmol/L) (n = 8). Photograph of EtBr gel shows PCR products in duplicate from 2 representative subjects (Mann-Whitney test).

DISCUSSION

Investigations in experimental animals and in humans have shown that the body fat content and different hormones regulate leptin gene expression and leptin secretion.[3] In the present in vitro study, we demonstrate for the first time a direct inhibitory effect of thyroid hormones on human adipose tissue. The inhibitory effect of thyroid hormones was found for basal and stimulated (dexamethasone) leptin secretion. In addition, the inhibitory effect was found for leptin secretion and leptin mRNA transcription. In the prospective in vivo study, we ensured that 60 μg T_3 per day was sufficient to produce a hyperthyroid state as assessed by laboratory data and confirmed by physiological measurement.[5] The T_3 level increased threefold after T_3 treatment but did not influence leptin levels. Adjusting leptin levels for the BMI did not change the results.

The most likely explanation for this discrepancy resides in a much higher concentration of free T_3 reaching the adipose tissue in vitro compared with the in vivo situation, but other possible explanations cannot be excluded. The circulating leptin probably results from differential production of leptin in different regions of adipose tissue,[19] and regions other than abdominal subcutaneous tissue may respond differently to T_3. The differential metabolic responses in different fat depots are well known for several hormones (insulin, catecholamines, and corticosteroids) but, to our knowledge, they have not been investigated for thyroid hormones. Other explanations for the discrepancy may be the removal of adipose tissue from the in vivo milieu, which may affect the degree of responsiveness to T_3 in vitro. In vitro, there is another extracellular milieu (concentrations of albumin, binding proteins, etc.), a longer distance of diffusion, and a missing nervous innervation and blood circulation, which may influence the concentrations of hormones necessary for biological effects. On the contrary, in vivo interference with other hormones or the central nervous system may obscure the regulation. In accordance with this, thyroid hormones increase the sensitivity to catecholamines by upregulation of adrenergic receptors, which tend to decrease leptin secretion. However, thyroid hormones may also induce hyperinsulinemia[20] and stimulate corticosterone secretion,[21] which counteract the adrenergic effects on leptin secretion.

The in vivo results are in agreement with a previous study in healthy men in acute hyperthyroidism, which also increased T_3 threefold.[22] The in vitro study is in accordance with a study by Fain and Bahouth[23] in which leptin mRNA expression decreased in isolated rat adipocytes and rat adipose tissue fragments after T_3 addition, whereas the reverse was found when thyroid hormone was added together with insulin (10 nmol/L). Yoshida et al[24] studied the mouse preadipocyte cell line 3T3-L1 and found stimulation by thyroid hormone. However, in mature preadipocytes, leptin expression is only a fraction (~1%)[25] of that in mature adipocytes, and in addition, the 3T3-L1 cell line is a clonal mouse preadipocyte cell line, making the study less relevant to human leptin physiology. Valcavi et al,[8] in hypothyroid patients, found a lower leptin level compared with controls and speculated as to whether normal thyroid hormone levels are needed to achieve adequate leptin gene expression. Our in vitro studies did not indicate that T_3 is necessary for leptin secretion in vitro, at least for 24-hour incubations. On the contrary, it seems that leptin is inhibited by thyroid hormone at high concentrations.

Whether the observed inhibitory effect of T_3 on adipose tissue leptin production in vitro has any physiological importance is unknown, although a T_3 level of about 20 nmol/L may be observed in severe thyrotoxicosis.[26] However, teleologically, the observed effect in vitro makes sense, since a state with high T_3 and high energy expenditure may signal to the brain, via low leptin, to increase the energy intake and to avoid excessive catabolism. Studies in humans with pathological levels of thyroid hormones are conflicting with regard to leptin levels.[8,27] Most studies do not find any change in leptin levels in hyperthyroid states,[10,28] whereas the results in hypothyroid subjects are contradictory.[8,10,29] This may be in part due to the study design, gender differences in study groups, and the level of hyperthyroidism or hypothyroidism. In addition, the duration of the hypothyroid state may influence leptin levels. Hypothyroid states are difficult to diagnose and the patients may vary substantially in the duration of symptoms, which may add to the inconsistent results. In conclusion, we report on the first study in which the direct effect of T_3 on human adipose tissue was examined in vitro. T_3 (>20 nmol/L) inhibited leptin production and leptin gene expression, whereas an elevation of T_3 corresponding to a moderately thyrotoxic state (T_3 5.25 ± 0.39 nmol/L) was without any impact on leptin in vivo. T_3 is possibly a negative physiological regulator of leptin levels in humans at high concentrations.

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