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Metabolic effects of chronic T3 administration in the hypothalamic paraventricular and ventromedial nucleus in male rats

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Abstract

Thyroid hormone is a key regulator of energy metabolism. Apart from its direct effects on peripheral metabolism, thyroid hormone exerts acute metabolic effects via distinct nuclei within the hypothalamus. Recently, we developed a method for chronic and local intrahypothalamic triiodothyronine (T3) administration in rats. The present study evaluated the metabolic effects of T3 delivered during either 7 or 28 days to the paraventricular or ventromedial nucleus of the hypothalamus (PVN or VMH). T3 administration for 7 days in the PVN decreased only plasma T3. There were no effects on body weight, food intake, plasma glucose concentrations, energy expenditure, locomotor activity and respiratory exchange rate (RER). In the liver and BAT, there were no changes in mRNA expression of genes involved in glucose metabolism and thermogenesis. T3 administration for 7 days in the VMH did not change any of these parameters. T3 administration for 28 days in the PVN decreased food intake without affecting body weight, glucose concentrations and body temperature. Liver and BAT gene expression was unaltered, except for decreased liver Dio1 mRNA. T3 administration for 28 days in the VMH did not affect liver and BAT mRNA expression, body weight, food intake and body temperature while blood glucose concentrations were slightly lower.

In conclusion, we showed that chronic T3 administration to the PVN or VMH does not affect energy metabolism in a major way. Our results imply that the effects of intrahypothalamic T3 administration on metabolism largely depend on the duration of treatment.

Key words

T3, PVN, VMH, energy expenditure, metabolism
**Metabolic effect of central T3**

**Introduction**

Thyroid hormone plays a critical role in energy metabolism. Hyperthyroidism is associated with increased metabolic rate and lower body weight, whereas hypothyroidism causes the reverse [1-4]. Recently, insight into the central, i.e., hypothalamic effects of thyroid hormone on energy metabolism has developed rapidly. Studies from our lab and other groups have shown a central role of short-term triiodothyronine (T3) administration in the regulation of liver glucose metabolism [5], brown adipose tissue (BAT) thermogenesis [6], food intake [7, 8] and cardiovascular function [9], through distinct nuclei in the hypothalamus. Within the hypothalamus, the hypothalamic paraventricular nucleus (PVN) is an integration center for autonomic output. A previous study of our laboratory showed that T3 administration in the PVN acutely stimulated hepatic glucose production through the sympathetic nervous system (SNS) [5]. The ventromedial hypothalamus of the hypothalamus (VMH) has been reported as a pivotal site for BAT thermogenesis and energy expenditure. Studies from the Lopez group showed increased BAT sympathetic nerve activity shortly after stereotactic microinjection of T3 in the VMH. This activation was mediated by an inhibition of AMP-activated protein kinase (AMPK) signaling in the VMH [6, 10]. Therefore, the metabolic phenotypes from hypo- and hyperthyroidism could be partly mediated via the hypothalamus and its neural outflow. However, although the acute metabolic effects in these studies are significant, their consequences upon prolonged T3 administration have not been demonstrated. We hypothesized that prolonged intrahypothalamic T3 administration results in neurally-mediated metabolic alterations as observed after acute hypothalamic T3 administration. We therefore recently developed a method to deliver T3 to the rat PVN or VMH for up to four weeks using slow-releasing T3 pellets [11]. The present study aimed to investigate the effects of chronic intrahypothalamic T3 administration in the PVN or the VMH, either for 7 or 28 days, on metabolic parameters, including body weight, food intake, energy expenditure, locomotor activity and blood glucose concentrations as well as on expression of genes in liver and BAT involved in glucose metabolism and thermogenesis, respectively.

**Materials and methods**

**Animals**

Male Wistar rats weighing 300-350 g (9-10 weeks) (Harlan Nederland, Horst, Netherlands) were housed individually in a 12h-12h light-dark cycle environment (lights on at
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07:00), with a room temperature of 23±2°C and 60±5% humidity. Chow and water were provided ad libitum. All procedures were approved by the Animal Care Committee of the Royal Netherlands Academy of Arts and Sciences.

T3 pellet implantation

Control beeswax pellets (cP) or T3-containing pellets (T3P) were prepared as previously reported [11]. In brief, 3, 3’, 5-Triiodo-L-thyronine (Sigma-Aldrich, Saint Louis, USA) was mixed with melted beeswax (Sigma-Aldrich, Saint Louis, USA) at a ratio of 1:9 (w/w) in a 70°C water bath. The mixture was packaged into a cylindrical pellet with a length of 1 mm using a 23G stainless cannula.

We performed experiments with T3 implantation for 7 days and for 28 days. The 7-day experiment was used for measuring metabolic parameters using calorimetric cages. Rats were allowed for a two-day recovery from surgery, two-day adaption to the metabolic cages for a stable output and three-day consecutive measurements. As hyperthyroid patients display metabolic alterations, including weight loss, after several weeks to months and the T3 pellets showed a continuously stable release of T3 for at least four weeks, we performed a 28-days experiment in order to test the long term effects of high hypothalamic T3 levels. After one week acclimatization in the facility, animals were anesthetized by either isoflurane (Abbott Laboratories Ltd, Maidenhead, UK) (7-day experiment) or by an intramuscular injection of a mixture of ketamine (80 mg/ml), xylazine (8 mg/ml) and atropine (0.1 mg/ml) (4:2:1, v:v:v) at a dosage of 1 ml/kg body weight (28-day experiment). cP’s or T3P’s were implanted bilaterally to the PVN (anteroposterior: -1.8 mm, lateral: 2.1 mm, ventral: -6.9 mm, angle: 10°) or VMH (anteroposterior: -2.3 mm, lateral: 2.2 mm, ventral: -8.1 mm, angle: 10°) region (Paxinos and Watson, 2005) using a standard Kopf stereotaxic apparatus.

Experiment setup

For the 7-day experiment, animals (n=32) were housed individually in calorimetric cages (Phenomaster/Labmaster system, TSE Systems GmbH, Bad Homburg, Germany) three days before pellet implantation. Basal metabolic parameters, i.e., oxygen consumption (VO₂) and carbon dioxide production (VCO₂) were measured continuously via an indirect open-circuit calorimetry. Respiratory exchange ratio (RER) and energy expenditure (EE) were calculated using the manufacturer’s formulas: RER = VCO₂/VO₂; EE = CV × VO₂, in which CV is calorific value (CV = 3.815 + 1.232 × RER). Locomotor activity was measured with ActiMot2 light beam frames (TSE Systems GmbH, Bad Homburg, Germany). Two days before surgery (day -2), blood samples were taken via a tail cut. At day 0, cP’s (n=7) or T3P’s (n=9) were implanted into either the PVN or the VMH under stereotactic guidance and animals were placed back in their previous calorimetric cages. Two hours after implantation, blood samples were taken via tail cuts. Animals were sacrificed at day 7.
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guidance and animals were placed back in their previous calorimetric cages. Two hours (2h) and 1, 4 and 7 days after implantation of the pellets, blood samples were taken via tail cuts. Animals were sacrificed at day 7.

For the 28-day experiment, animals (n=40) received cP’s (n=16) or T3P’s (n=24) in the PVN or the VMH. During surgery, part of the animals (n=16) received a nano-thermo logger (Star-Oddi Ltd., Iceland) subcutaneously (SC) just above the interscapular BAT for continuous body temperature recording. Blood was taken via tail cuts at day -2, day 7, 14 and 28 days after pellet implantation. Animals were sacrificed at day 28.

At the end of all the experiments, animals were decapitated. Liver and BAT were snap frozen in liquid nitrogen and stored in -80°C until further use. Brains were removed and frozen on dry ice. The hypothalamus was cut at 20 μm coronal sections using a cryostat (Leica CM1950) and stained with cresyl violet in order to check pellet placement.

Blood and plasma analysis

Blood samples were taken using heparin coated capillary Microvette (Sarstedt AG & Co, Nümbrecht, Germany) by tail cut. Blood glucose concentrations were determined immediately by a glucose meter (FreestyleTM, Abbott, the Netherlands) during the sampling. At the end of each experiment, animals were decapitated and trunk blood was collected. The plasma samples were collected after centrifugation at 4000rpm for 15 min and stored at -20°C. Plasma T3 and T4 concentrations were determined using an in-house RIA (inter-assay variation T3, 6.2% and T4, 7.3%; intra-assay variability T3, 3.6% and T4, 6.6%) [12, 13]. Plasma TSH was determined by a Chemiluminescent Immunoassay, using the Immulite 2000 and a rat specific standard (Siemens, Munich, Germany) with an inter-assay variation of ±9% and an intra-assay variation of 3-4%. The samples of one experiment were measured in one run to prevent inter-assay variation.

RNA isolation and quantitative PCR (qPCR)

Total RNA from BAT and liver was isolated using Machery-Nagel RNA isolation kit (MACHEREY-NAGEL GmbH & Co. KG, Düren, Germany) and RNA yield was determined using the DeNovix DS-11 Spectrophotometer (DeNovix, Wilmington, Delaware USA). The cDNA synthesis was performed with equal RNA input using the Transcriptor First Strand cDNA synthesis kit (Roche Molecular Biochemicals, Mannheim, Germany). As a control for genomic DNA contamination, we included a cDNA synthesis reaction without reverse transcriptase. Quantitative PCR was performed using the LightCycler 480 (Roche Molecular Biochemicals) and SensiFAST™ SYBR No-ROX mix (Bioline, GmbH, Germany). The primers used for qPCR are listed in Table 3.1. Quantification was performed using
the LinReg software. PCR efficiency was checked individually and samples with a deviation of more than 5% of the mean were excluded from analysis. Values were expressed according the geometric mean of Gapdh and Hprt.

Table 3.1 Primers used for qPCR

<table>
<thead>
<tr>
<th>Gene</th>
<th>symbol</th>
<th>Forward (5’-3’)</th>
<th>Reverse (5’-3’)</th>
<th>Products length (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>glyceraldehyde-3-phosphate dehydrogenase</td>
<td>Gapdh</td>
<td>TGAACGGGAAGCTCAGCTGG</td>
<td>TCCACCACCCCTGTGGTCGTA</td>
<td>306</td>
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<tr>
<td>hypoxanthine guanine phosphoribosyl transferase</td>
<td>Hprt</td>
<td>GCAGTACAGCCCCAAAAAACAACTGG</td>
<td>ACAAAGTCTGGGCTGCTATCCAA</td>
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</tr>
<tr>
<td>Deiodinase type 1</td>
<td>Dio1</td>
<td>GAA GTG CAA CGT CTG GGA TT</td>
<td>CTGGCGAACCTGTTACCTTCTC</td>
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</tr>
<tr>
<td>Deiodinase type 2</td>
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<td>TCCTGGAGCCGTTCTCCTG</td>
<td>CATAGCTACGTGTCATTGTG</td>
<td>78</td>
</tr>
<tr>
<td>Peroxisome proliferator-activated receptor gamma, coactivator 1 alpha</td>
<td>Pgc1a</td>
<td>CAAATGATGCAGCGGTCGT</td>
<td>GTGTGAGGAGGGCTGCGTT</td>
<td>195</td>
</tr>
<tr>
<td>Peroxisome proliferator-activated receptor gamma</td>
<td>Pparg</td>
<td>CAGGAAAGACACAGCAGAAAATCA</td>
<td>GGGGTGTGATGTTTTCTGGA</td>
<td>95</td>
</tr>
<tr>
<td>Phosphoenolpyruvate carboxykinase 1</td>
<td>Pepck</td>
<td>GTGCCCTCTGTACGAACTGGT</td>
<td>GGTGTGCGATGATGACGGGTTTCTG</td>
<td>116</td>
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<tr>
<td>Fatty acid synthase</td>
<td>Fasn</td>
<td>CTGAGGTTGGTCCGATTACAACC</td>
<td>GCCCTACCGTGATCACCCTGTT</td>
<td>163</td>
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<tr>
<td>Uncoupling protein 1</td>
<td>Ucp1</td>
<td>AATCAGCTCTTGCTCCCTGACA</td>
<td>GTTGTGCTGTTGATTCTGCACTGTA</td>
<td>181</td>
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<tr>
<td>Adrenergic receptor, beta 3</td>
<td>Adrb3</td>
<td>CTTCCCAGCTAGCCTGTT</td>
<td>CTTGCTAGATCTCACTTGGGA</td>
<td>110</td>
</tr>
</tbody>
</table>

**Statistical analysis**

Data are expressed as mean ± standard error of the mean (SEM). Differences between two groups were analyzed using an independent two-tailed Student’s t test. When data were not normally distributed, the independent non-parametric Mann-Whitney U test was used. The changes at different time points were evaluated by two-way ANOVA with repeated measurements followed by Sidak post hoc analysis. Statistical significance was defined at a level of $p<0.05$. All analyses were performed using Graph Pad Prism 6.05.
Results

Effect of T3 administration in the PVN and VMH on thyroid hormones concentrations

Our previous study showed that 4-week T3 administration in the PVN reduced plasma T3 and T4 concentrations while TSH was not affected, whereas 4-week T3 administration in the VMH did not change plasma T3, T4 and TSH [11]. In the present study we determined the metabolic effects of T3 administration in the PVN or VMH either for a relatively short period (7 days) or a longer term period (28 days). We also measured serum thyroid hormone (TH) levels after 7 days’ exposure to T3 in the PVN or VMH. Placement of T3-containing pellets in the PVN for 7 days lowered plasma T3 concentrations (ANOVA, $P_{\text{treatment}} < 0.001$). Lowest plasma T3 levels were reached at day 1 ($P < 0.001$) and almost returned to control levels at day 7 (Figure 3.1A). Plasma T4 and TSH concentrations did not differ between T3 treatment and control rats (Figure 3.1C, E). Placement of T3-containing pellets in the VMH for 7 days did not change plasma T3 concentrations (Figure 1B), while T4 concentrations were slightly lower in the T3P group (ANOVA, $P_{\text{treatment}} = 0.038$) (Figure 3.1D). Plasma TSH concentrations did not differ between control and T3 treatment (Figure 3.1F).

Chronic effect of T3 administration in the PVN

Effects of T3 administration in the PVN on body weight, food intake, glucose concentrations and body temperature

Administration of T3 in the PVN for 7 days did not change body weight, daily and total food intake and plasma glucose concentrations (Figure 3.2A, C and E). Administration of T3 in the PVN for 28 days did not change body weight and daily food intake, but total food intake was lower in the T3P group (Figure 3.2B and D). Plasma glucose concentrations and body temperature were not different during 28 days of T3 administration in the PVN (Figure 3.2F, G).

Effect of T3 administration in the PVN for 7 days on energy metabolism

Animals were housed in the metabolic cages throughout the 7-day experiment in order to record energy expenditure (EE), respiratory exchange rate (RER) and locomotor activity. Administration of T3 in the PVN did not affect EE, RER and locomotor activity (Figure 3.3A, B and C).

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Effect of T3 administration in the PVN on gene expression in liver and BAT

T3 administration in the PVN for 7 days did not change mRNA expression of liver Dio1, Pgc1a, Pparg, Fasn and Pepck (Figure 3.4A). There were also no changes in mRNA expression of Pgc1a, Pparg, Dio2, Ucp1 and Adrb3 in BAT (Figure 3.4C). After 28 days, liver Dio1 mRNA expression was decreased and Pparg mRNA expression was increased in T3P group (Figure 3.4B). Pgc1a, Pparg, Dio2, Ucp1 and Adrb3 mRNA expression in BAT was not significantly different between cP and T3P (Figure 3.4D).

Pellets in the PVN for 7 days

Pellets in the VMH for 7 days

Figure 3.1 Plasma thyroid hormones concentrations after placement of a T3-containing pellet in the PVN (A and C) or VMH (B and D). Plasma T3 and T4 concentrations were determined 2 days before, 2 hours, 1 day, 4 days and 7 days after pellet placement in the PVN (A and C) or the VMH (B and D). TSH concentration was measured 7 days after pellet placement in the PVN (E) or the VMH (F). *** P<0.001 cP vs T3P. N=6-7 per group.
Effect of T3 administration in the PVN on gene expression in liver and BAT

T3 administration in the PVN for 7 days did not change mRNA expression of liver Dio1, Pgc1a, Pparg, Fasn and Pepck (Figure 3.4A). There were also no changes in mRNA expression of Pgc1a, Pparg, Dio2, Ucp1 and Adrb3 in BAT (Figure 3.4C). After 28 days, liver Dio1 mRNA expression was decreased and Pparg mRNA expression was increased in T3P group (Figure 3.4B). Pgc1a, Pparg, Dio2, Ucp1 and Adrb3 mRNA expression in BAT was not significantly different between cP and T3P (Figure 3.4D).

Figure 3.1 Plasma thyroid hormones concentrations after placement of a T3-containing pellet in the PVN (A and C) or VMH (B and D). Plasma T3 and T4 concentrations were determined 2 days before, 2 hours, 1 day, 4 days and 7 days after pellet placement in the PVN (A and C) or the VMH (B and D). TSH concentration was measured 7 days after pellet placement in the PVN (E) or the VMH (F). ***, P<0.001 cP vs T3P. N=6-7 per group.

Figure 3.2 Body weight (A and B), food intake (C and D), glucose concentrations (E and F) and body temperature (G) after placement of a T3-containing pellet in the PVN. Pellets were placed for 7 days (A, C and E) and 28 days (B, D, F and G). Total food intake is shown as an embedded bar graph along with daily food intake (C and D). *, P<0.05, N=6-7 per group in 7 days experiment; N=8-10 for 28 days experiment. N=3 for temperature data.
Chronic effect of T3 administration in the VMH

Effect of T3 administration in the VMH on body weight, food intake, glucose concentrations and body temperature

Administration of T3 in the VMH for 7 days did not affect body weight, total food intake and glucose concentrations (Figure 3.5A, C and E). Administration of T3 for 28 days did not result in changes in body weight, daily and total food intake (Figure 3.5B and D), while plasma glucose concentrations were slightly lower in T3 treated rats (ANOVA, \( P_{\text{treatment}} =0.047 \)) (Figure 3.5F). Body temperature was not affected (Figure 3.5G).

Figure 3.3 Energy metabolism after placement of a T3 containing pellet in the PVN for 7 days. Energy expenditure (EE) (A), respiratory exchange rate (RER) (B) and locomotor activity (C) were monitored 2 days before until 6 days after pellet placement. N=6-7 per group.


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**Effect of T3 administration in the VMH for 7 days on energy metabolism**

Administration of T3 in the VMH for 7 days did not change EE, RER and locomotor activity (Figure 3.6A, B and C).

**Effect of T3 administration in the VMH on gene expression in liver and BAT**

T3 administration in the VMH for 7 and 28 days did not change the mRNA expression of liver Dio1, Pgc1a, Pparg, Pepck and Fasn and BAT Pgc1a, Pparg, Dio2, Ucp1 and Fasn (Figure 3.7).

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**Figure 3.4** Gene expression in liver (A and B) and brown adipose tissue (BAT) (C and D), determined by qPCR, after placement of T3-containing pellets in the PVN for 7 or 28 days. N=6-10 per group. **, P<0.01

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**Discussion**

Recent work has established a significant role for T3 in the regulation of peripheral energy metabolism via the central nervous system. We recently developed a rat model for chronic and local intrahypothalamic T3 administration. In the present study, we investigated the metabolic effects of chronic (7 and 28 days) T3 administration in the PVN and VMH.
Figure 3.5 Body weight (A and B), food intake (C and D), glucose concentrations (E and F) and body temperature (G) after placement of T3-containing pellets in the VMH. Pellets were placed for 7 days (A, C and E) or 28 days (B, D, F and G). Total food intake is shown as an embedded bar graph along with daily food intake (C and D). N=6-7 per group in 7 days experiment; N=8-10 for 28 days experiment. N=3 for temperature data.
The PVN is the key nucleus for regulation of the hypothalamic-pituitary-thyroid (HPT) axis set point. T3 negatively modulates the HPT axis via suppressing TRH production in the hypophysiotropic thyrotropin-releasing hormone (TRH) neurons in the PVN [14]. As expected, T3 administration in the PVN for 7 days decreased plasma T3 and T4 concentrations, which is in agreement with our previous results obtained after 28 days of T3 administration [11]. In line with this, the T3 responsive gene *Dio1* in liver showed lower mRNA expression after 28 days of T3 administration in the PVN. T3 administration in the PVN for 7 days did not change daily food intake significantly. However, total food intake after 28 days was significantly decreased. Thyroid hormone is known to stimulate food intake and hypothyroidism results in reduced appetite [15, 16]. Thus, the lower circulating thyroid hormones observed after 28 days of T3 administration in the PVN might explain the decrease of daily food intake. Of note, it was also shown that T3 stimulates food intake directly at the hypothalamic level [7, 15, 17, 18] and the PVN is
one of the essential integration centers for hormonal modulation of food intake and body weight [19, 20]. As we showed earlier that hypothalamic T3 levels are markedly increased for at least 28 days as a result of implanting T3-containing pellets [11], diminished food intake is therefore difficult to reconcile with a direct stimulating effect of T3 on food intake at the level of the PVN in our experimental setting.

Thyroid hormone is a critical regulator for glucose metabolism [21]. Fasting induced hypoglycemia is associated with decreased plasma thyroid hormone levels [22, 23]. A previous study of our lab showed that acute T3 administration via microdialysis in the PVN increased blood glucose concentrations and hepatic glucose production via the sympathetic nervous system [5]. However, in the present experiments we did not find changes in blood glucose concentrations after either 7 days or 28 days of T3 administration in the PVN. Considering the lower circulating TH concentrations observed during T3 administration in the PVN, the stimulating effect of hypothalamic T3 on glucose might be counteracted by the lower plasma T3 concentrations. Indeed, an essential transcriptional factor \( Ppar \) that restrains glucose production [24] was remarkably increased in the liver after T3 administration in the PVN, whereas \( Pepck \) tended to be decreased, suggesting a stimulation of glucose storage.

Figure 3.7 Gene expression in liver (A and B) and brown adipose tissue (BAT) (C and D), determined by qPCR, after placement of a T3-containing pellet in the VMH. N=6-10 per group.
Administration of T3 in the VMH either for 7 days or 28 days did not change plasma T3 and T4 concentrations. The differential effects of long-term T3 administration in the PVN and VMH on the HPT axis confirm our previous study showing that we are able to deliver T3 selectively to either the PVN or the VMH [11]. The VMH is an important integration center for energy metabolism. A previous study showed that a single injection of T3 in the VMH markedly increased food intake after one hour without altering energy expenditure [18]. In contrast, Lopez et al. showed that T3 injection in the VMH did not affect food intake after one hour but significantly increased sympathetic nerve activity in BAT [6]. In the present study, T3 was delivered to the VMH constantly for 7 or 28 days. Surprisingly, we did not observe changes in food intake and body weight upon chronic T3 administration in the VMH. Varela et al. showed that T3 administration in the arcuate nucleus (ARC) of rats significantly increased food intake [7]. Moreover, 24-h fasting induced hyperphagia in mice was shown to be associated with increased T3 availability in the ARC [8]. Thus, the hyperphagia seen in hyperthyroidism may be mediated primarily through the ARC, probably involving mammalian target of rapamycin (mTOR) and the UCP2 pathway [7, 8]. Hyperthyroidism results in increased food intake but reduced bodyweight probably due to increased energy expenditure [4]. We however, did not observe any change in energy expenditure, locomotor activity and body weight in rats after T3 administration in the VMH for 28 days. Our results therefore suggest that the effects of TH during chronic hyperthyroidism on energy expenditure and body weight are probably not solely mediated via the VMH. Thyroid hormone is important for sympathetic regulation and thermogenesis [1, 25, 26]. Central T3 administration by intracerebroventricular (ICV) injection showed increased sympathetic nerve activity in BAT, inducing a marked thermogenic effect [6, 10]. However, T3 administration in the VMH for 28 days in our study did not change body temperature. In line, gene expression in BAT did not show changes in Dio2 and Ucp1, both involved in heat production in BAT. A recent study showed that thyrotoxicosis induced by two-week T4 administration in mice increased body temperature but did not affect either BAT thermogenesis or BAT UCP1 content, suggesting that BAT is not involved in thyroid hormone induced hyperthermia [27]. In fact, a prolonged hyperthyroid state is associated with reductions of both BAT sympathetic activity and UCP1 dependent thermogenesis [28, 29]. Moreover, TH also plays an essential role in tail vascular heat conservation and dissipation [30, 31], a mechanism that may counteract the hyperthermic effects of T3. To add more complexity, another study with ICV T3 administration to mice pre-acclimated to 18°C showed increased daily body temperature but elevated energy expenditure only in the dark phase [32], suggesting also a circadian influence on T3-mediated energy metabolism. We did not find changes in energy expenditure as seen in this ICV study, however,
one should notice that our T3 pellet released T3 only to the targeted nuclei \([11]\) while an ICV injection of T3 will certainly have indirect actions via other nuclei that e.g. project to the VMH, which could be a prerequisite for the T3 induced induction of BAT thermogenesis.

Of note, it is unclear whether damage to the hypothalamic nuclei or neural connections by the pellets implantation might mask possible metabolic effect of central T3. However, we compared plasma T3, T4 and glucose levels of naive and untreated rats with a group that received a blank pellet and did not observe any differences. Furthermore, the rats that received a blank pellet did not display altered deiodinase expression locally, which is a measure for tissue damage or inflammation. There were also no differences in food intake, body weight and gene expression in the VMH (e.g., Dio3, Hairless, Pacap, Fasn) between intact and control pellet group. These data indicate no damage and side effects resulting from the pellet implantation per se \((11)\) and Supplementary Figure S1 and S2).

In conclusion, T3 administration in the PVN for 7 days did not change body weight, food intake, and energy expenditure. T3 administration in the PVN for 28 days decreased total food intake but did not affect body weight and glucose concentrations. Administration of T3 in the VMH for 7 days did not change body weight, food intake and energy expenditure. T3 administration in the VMH for 28 days resulted in changes in blood glucose concentrations but unchanged body weight, food intake and body temperature. We conclude that chronic T3 administration to the PVN or VMH does not affect energy metabolism. Our results imply that the effects of intrahypothalamic T3 administration on metabolism depend on the duration of treatment in a major way.

Acknowledgement

We would like to thank the staff of the laboratory of Endocrinology for measuring serum thyroid hormones.

References

We conclude that chronic T3 administration to the PVN or VMH does not affect energy expenditure. T3 administration in the VMH for 28 days resulted in changes in blood total food intake but did not affect body weight and glucose concentrations. (Supplementary Figure S1 and S2).

...damage and side effects resulting from the pellet implantation per se ([11] and Hairless, Pacap, Fasn). These data indicate no differences in food intake, body weight and gene expression in the VMH (e.g., Dio3, Dio2, and Dio1). However, we compared plasma T3, T4 and glucose levels of naive and untreated rats to assess the extent to which the pellets implantation might mask possible metabolic effect of central T3. In conclusion, T3 administration in the PVN for 7 days did not change body weight, food intake or glucose levels.

We would like to thank the staff of the laboratory of Endocrinology for measuring serum total triiodothyronine (T3), total thyroxine (T4), and glucose levels.

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