Presence and regulation of D1 and D2 deiodinases in rat white adipose tissue

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Abstract

Thyroid hormones regulate adipogenic differentiation, lipogenic and lipolytic metabolism and mitochondrial activity in adipose tissue. T3 levels in tissues are regulated by the deiodinase enzymes.

Objective: To study the activity and mRNA expression of the 5' outer-ring deiodinases (D1 and D2) and thyroid hormone concentrations in rat white adipose tissue (WAT), where only D1 activity had been described.

Methods: Control, thyroidectomized and thyroid hormone treated rats were used. D1 and D2 mRNA were determined in WAT by qRT-PCR using Taqman probes, D1 and D2 activities were determined using rT3 and T4 as substrates. T4 and T3 were measured by radioimmunoassay in plasma, liver and adipose tissue.

Results: D1 and D2 mRNAs are present in epididymal rat WAT with similar abundance, which are 7% of the D2 mRNA levels in BAT and 1% that of D1 in liver. The Kms in WAT are 40 nM T4 for D2 and 0.35 μ M rT3 for D1. Both D1 and D2 are regulated in rat epididymal WAT by thyroidal status. T4 and T3 concentrations in plasma, liver and WAT decreased after thyroidectomy (Tx) and recovered after treatment with T4+T3. Both D1 and D2 mRNA increased in WAT from Tx rats and T4+T3 treatment inhibited them, especially D2 mRNA. D1 activity did not changed with thyroidal status, while D2 activity was inhibited by T4+T3.

Conclusions: the presence of both deiodinases in WAT suggests important roles in regulating T3 bioavailability for adipose tissue function and regulation of lipid metabolism and thermogenesis.

Keywords: epididymal adipose tissue, thyroid hormones, outer-ring deiodinases.

52 **List of abbreviations:**

53 BAT: brown adipose tissue; D1: type1 deiodinase; D2: type 2 deiodinase; D3: type3
54 deiodinase; DTT: dithiothreitol; HEPES: 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid;
55 Km: Michaelis-Menten constant; PCR: polymerase chain reaction; PTU: 6-propyl-2-
56 thiouracil; qRT-PCR: quantitative Real-time PCR; rT3: reverse triiodothyronine; T3:
57 triiodothyronine; T4: thyroxine; Tx: thyroidectomized; UCP1: uncoupling protein 1; Vmax:
58 maximal velocity; WAT: white adipose tissue.

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Introduction

Deiodinases are selenoenzymes that regulate thyroid hormone concentrations in tissues. Two isoenzymes, D1 and D2, catalyze 5'deiodination producing T3 from T4. D1 is present in liver, kidney and thyroid. D1 Km is 0.2-0.5 μ M [1], is inhibited by PTU, increases in hyperthyroidism and decreases in hypothyroidism, except thyroid D1 which increases in hypothyroidism, by thyrotropin stimulation [2]. D2 is present in pituitary, brain, BAT and other tissues, is not inhibited by PTU, its Km in nanomolar range. D2 increases in hypothyroidism and produces T3 for local needs. D3, the main T3 degradation pathway, catalyzes inner ring deiodination of T4 and T3, leading to inactive metabolites.

D2 is essential for the regulation of T3 availability during specific events of development, in the ear, retina, brain or BAT. T3 is required for the differentiation program of adipocytes [3], regulating the expression of genes involved in the differentiation and metabolism of adipose tissue. Most studies demonstrating the importance of deiodinases, specifically D2, have been performed in BAT, where T3, locally produced by D2, is important for full thermogenesis, UCP1 expression and lipogenesis [4-7]. So far, no studies have been performed on D1 or D2 abundance in WAT, its hormonal regulation or potential role in different metabolic processes, though D1 activity in WAT was found when D2 was reported in BAT [8]. Some studies have shown induction of brown adipocytes in inguinal WAT depots, using UCP1 and D2 as markers [9, 10]. In this respect epididymal WAT is considered "pure" WAT, never converted into BAT even under extreme cold exposure, as opposed to inguinal WAT considered "convertible" adipose tissue [11]. In humans, WAT represents 15-20% of the body weight in lean subjects and this percentage is much higher in obese people (50%). Thus, WAT might represent one of the largest pools of thyroid hormones. In addition, if WAT can be converted into BAT (inducing UCP1), WAT represents a potential therapeutic

target to increase energy expenditure in obesity. Here we studied the presence and regulation by thyroid status of D1 and D2 activities and mRNA levels in rat WAT.

Materials and Methods

Animals and treatments

Protocols following the European Community guidelines were approved by our ethic Committee. Male Wistar rats were divided in 3 groups: Control, Tx and Tx rats treated with T4+T3 (2.4 µg T4+0.4 µg T3/day/100 g body weight) as described [12]. Plasma and organs were dissected out and frozen on dry ice. Epididymal WAT was carefully dissected avoiding the reproductive male organs.

Analytical procedures

Determination of D1 and D2 activities. D1 and D2 activities were assayed in WAT homogenates (1:8, weight/volume) using 0.32 M sucrose, 10 mM Hepes and 10 mM DTT. Homogenates were centrifuged 5 minutes at 1000 rpm to separate the upper lipid cake, the infranant was used to measure deiodinase activities.

D1 activity: 60.000 cpm [¹²⁵I]-rT₃/sample, 100 nM rT₃ and 5 mM DTT, for 1 h at 37 C using 20-30 µg protein/ 100 µl [13]. D2 activity: 100.000 cpm [¹²⁵I]-T₄/tube, 2 nM T₄+ 1 µM T₃, 20 mM DTT and 1 mM PTU/100 µl, using the same conditions and protein [14]. For kinetic analysis: 2-500 nM rT₃ and 5 mM DTT were used for D1 and 1-50 nM T₄ and 20 mM DTT for D2.

D1 and D2 mRNA

Total RNA was extracted using the RNeasy lipid tissue (Quiagen). D1 and D2 mRNAs were measured by qRT-PCR, using Taqman probes (Rn00572183m1, Rn00581867m1, Applied Biosystems). After normalization to 18S rRNA, the fold-change in mRNA expression was calculated by the $2^{-\Delta\Delta C_t}$ method.

Determination of thyroid hormone concentrations.

Thyroid hormone concentrations were determined by RIA in plasma, liver and WAT, after extraction and purification [15]. High specific activity T₄, rT₃ and T₃ labelled with [¹²⁵I] were synthesized as described [15].

Statistical analysis

Mean values ($\pm$ SEM) are given. Significant differences were achieved by one-way ANOVA analysis.

Results

Presence and characterization of D1 and D2 in epididymal rat WAT

We first identified D1 in WAT, using PCR amplification and gel separation (not shown). Then, we did titration curves using qRT-PCR using Taqman probes (**Figure 1A**), confirming that D1 and D2 mRNA were present in rat epididymal WAT being D2 mRNA abundance higher than D1 mRNA (approx. double). WAT D1 mRNA abundance was less than 1% of liver D1 and WAT D2 mRNA abundance was about 7% of BAT D2 (Figure 1B, 1C).

We then analyzed D1 and D2 activities in WAT. First, the kinetic characteristics were determined; for D2: $K_m=40$ nM T₄ and $V_{max}= 3$ pmol/h/mg protein and for D1: $K_m= 0.35$ μ M rT₃ and $V_{max}= 6-18$ pmol/h/mg protein (C and Tx rats, respectively). D1 activity was higher in perirenal WAT; D2 activity was similar in perirenal and epididymal WAT and lower in subcutaneous WAT (results not shown).

Regulation of D1 and D2 in rat WAT by thyroid status

Deiodinases are regulated by thyroid status in most tissues. To study this regulation we used control, Tx and T₄+T₃-treated rats. The thyroidal status was checked measuring T₄ and T₃ concentrations in plasma, liver and WAT. **Figure 2** shows that T₄ and T₃ decreased after thyroidectomy in all samples. Treatment of Tx rats with T₄+T₃ reverted T₄ to control values

in plasma and liver, while WAT T4 doubled the values in control rats, pointing to a preferential T4 uptake in WAT. The treatment also increased T3 in all cases, reaching control values in WAT and plasma, while liver T3 concentrations did not fully recovered.

D1 and D2 mRNA increased in WAT from Tx rats (**Figure 1D, 1E**), and T4+T3 treatment inhibited them to control values for D1, and to less than 50% of control values for D2 mRNA. Opposite to the mRNA responses no variation was observed in D1 and D2 activities, except for D2 activity that was inhibited in the T4+T3 group.

Discussion

D1 activity was identified in WAT in 1983 together with D2 activity in BAT [8], establishing clear differences between BAT and WAT. Then, a full UCP1 expression was associated to T3 produced by D2 in BAT [4] and this was recently confirmed in the D2 knockout mice [7].

Herein, we describe that D2 is also present in WAT, both at mRNA and activity levels. We used epididymal WAT, the purest of all WAT locations, never converted into BAT under cold exposure; in contrast, inguinal fat, is considered “convertible” adipose tissue, that is transformed into BAT under several stimuli [11, 16]. Other WAT locations (perirenal, periuterine) are considered a mix of BAT and WAT in terms of UCP1 induction [17]. UCP1 levels in epididymal WAT are near detection limits by qRT-PCR and less than 5% those in inguinal WAT (unpublished). Therefore it is unlikely that D2 could come from residual BAT cells present in epididymal WAT.

Despite the 1983 report, WAT D1 had not been further studied, possibly because lipids cause technical difficulties, which we solved by improving the RNA isolation using a specific kit to avoid lipids and specific Taqman probes. Regarding D1 and D2 activities, we

characterized them in terms of their kinetic characteristics which were in the same ranges as in other tissues [1].

Thyroid status, a main regulator of deiodinases, was studied in this work. Both deiodinases mRNAs were up-regulated in hypothyroidism. WAT D2 increased as expected in hypothyroidism. WAT D1 also increased, responding to hypothyroidism as thyroid D1 [2]. Regarding deiodinase activities, WAT D2 followed a pattern similar to D2 mRNA, as it tended to increase in hypothyroidism and decreased in T4+T3-treated rats, reflecting the high WAT T4 concentrations. We showed recently increased D2 mRNA in parallel to D2 activity levels in human subcutaneous fat [18]. D1 activity did not change among groups in our study. Other studies have shown discrepancies between D1 and/or D2 mRNA and activity levels [19], probably due to posttranscriptional modifications.

Recently, D1 activity in WAT was related to adiposity, increasing by high-fat diets and by leptin, and decreasing under caloric restriction [20]. Other possible regulators (hormones, nutrients) remain to be identified, as we have only explored the role of thyroid hormones. D1 and D2 may have distinct functions in WAT, regulating lipogenesis and lipolysis or the expression of genes and providing the T3 required for specific functions, including the conversion of WAT into BAT under specific conditions, which would increase energy expenditure. Each enzyme may have specific functions.

Conclusions

D1 and D2 activities and mRNAs are present in rat WAT. Thyroidal status regulates D1 and D2 mRNA in vivo, both increasing in hypothyroid rats. No changes are found in deiodinase activities, except for the D2 inhibition by T4+T3.

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190 **Authors contribution**

191 Both authors contributed equally to the study. MJO wrote the manuscript.

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Legends to Figures

Figure 1. Ct values for 18S rRNA, D1 and D2 mRNAs from epididymal rat WAT (panel A). Relative expression of D1 (panel B) and D2 mRNAs (panel C) versus liver D1 and BAT D2 mRNAs, respectively. Panels D to G show D1 and D2 mRNAs and activities in WAT from control (C), thyroidectomized (Tx) and T4+T3 treated thyroidectomized rats. Values are means $\pm$ SEM. * $P < 0.05$ vs C, # $P < 0.05$ vs Tx. (n=4-5/ group).

Figure 2. T4 and T3 concentrations in plasma, liver and epididymal WAT from control (C), thyroidectomized (Tx) and T4+T3 treated thyroidectomized rats. Values are means $\pm$ SEM. * $P < 0.05$ vs C, # $P < 0.05$ vs Tx. (n=4-5 /group)

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