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Preliminary Report

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. Triiodothyronine (T3) levels in tissues are regulated by the deiodinase enzymes. The objective was to study the activity and messenger RNA (mRNA) expression of the 5′ outer-ring deiodinases (type 1 [D1] and type 2 [D2] deiodinase) and thyroid hormone concentrations in rat white adipose tissue (WAT), where only D1 activity had been described. Control, thyroidectomized, and thyroid hormone–treated rats were used. Type 1 and type 2 deiodinase mRNAs were determined in WAT by quantitative real-time polymerase chain reaction using Taqman probes; D1 and D2 activities were determined using reverse T3 and thyroxine (T4) as substrates. Thyroxine and T3 were measured by radioimmunoassay in plasma, liver, and adipose tissue. Type 1 and type 2 deiodinase mRNAs are present in epididymal rat WAT with similar abundance, which is 7% of the D2 mRNA levels in brown adipose tissue and 1% of D1 in liver. The Michaelis-Menten constants in WAT are 40 nmol/L T4 for D2 and 0.35 μmol/L reverse T3 for D1. Both D1 and D2 are regulated in rat epididymal WAT by thyroidal status. Thyroxine and T3 concentrations in plasma, liver, and WAT decreased after thyroidectomy and recovered after treatment with T4 + T3. Both D1 and D2 mRNAs increased in WAT from thyroidectomy rats; and T4 + T3 treatment inhibited them, especially D2 mRNA. Type 1 deiodinase activity did not change with thyroidal status, whereas D2 activity was inhibited by T4 + T3. 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.

1. Introduction

Deiodinases are selenoenzymes that regulate thyroid hormone concentrations in tissues. Two isoenzymes, type 1 (D1) and type 2 (D2) deiodinase, catalyze 5′deiodination, producing triiodothyronine (T3) from thyroxine (T4). Type 1 deiodinase is present in liver, kidney, and thyroid. Type 1 deiodinase Michaelis-Menten constant (Km) is 0.2 to 0.5 μmol/L [1], is inhibited by 6-propyl-2-thiouracil (PTU), increases in hyperthyroidism, and decreases in hypothyroidism, except thyroid D1 that increases in hypothyroidism, by thyrotropin stimulation [2]. Type 2 deiodinase is present in pituitary, brain, brown

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adipose tissue (BAT), and other tissues and is not inhibited by PTU; its Km is in the nanomolar range. Type 2 deiodinase increases in hypothyroidism and produces T3 for local needs. Type 3 deiodinase, the main T3 degradation pathway, catalyzes inner-ring deiodination of T4 and T3, leading to inactive metabolites.

Type 2 deiodinase is essential for the regulation of T3 availability during specific events of development in the ear, retina, brain, or BAT. Triiodothyronine 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, uncoupling protein 1 (UCP1) expression, and lipogenesis [4-7]. So far, no studies have been performed on D1 or D2 abundance in white adipose tissue (WAT), its hormonal regulation, or potential role in different metabolic processes, although 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, which is considered "convertible" adipose tissue [11]. In humans, WAT represents 15% to 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 messenger RNA (mRNA) levels in rat WAT.

2. Materials and methods

2.1. 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 per day per 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.

2.2. Analytical procedures

2.2.1. Determination of D1 and D2 activities

The D1 and D2 activities were assayed in WAT homogenates (1:8, wt/vol) using 0.32 mol/L sucrose, 10 mmol/L 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, and 10 mmol/L dithiothreitol (DTT). Homogenates were centrifuged for 5 minutes at 1000 rpm to separate the upper lipid cake; the infranatant was used to measure deiodinase activities.

Type 1 deiodinase activity: 60 000 cpm [^{125}I]-reverse T3 (rT3)/sample, 100 nmol/L rT3 and 5 mmol/L DTT for 1 hour at 37°C using 20 to 30 μg protein per 100 μL [13]. Type 2 deiodinase activity: 100 000 cpm [^{125}I]-T4/tube, 2 nmol/L T4 + 1 μmol/L T3, 20 mmol/L DTT, and 1 mmol/L PTU per 100 μL, using the same conditions and protein [14]. For kinetic analysis: 2 to 500 nmol/L rT3 and 5 mmol/L DTT were used for D1 and 1 to 50 nmol/L T4 and 20 mmol/L DTT for D2.

2.3. D1 and D2 mRNA

Total RNA was extracted using the RNeasy lipid tissue (Qiagen, Madrid, Spain). Type 1 and type 2 deiodinase mRNAs were

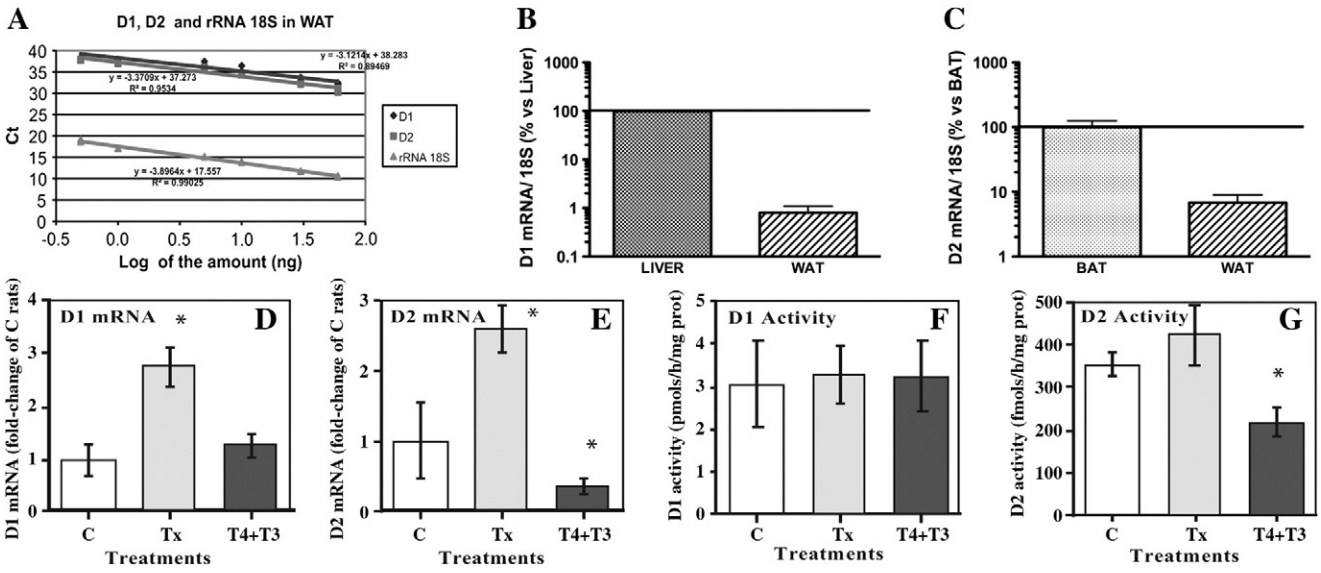

Fig. 1 – The Ct values for 18S rRNA and D1 and D2 mRNAs from epididymal rat WAT (A). Relative expression of D1 (B) and D2 mRNAs (C) vs liver D1 and BAT D2 mRNAs, respectively. D to G, D1 and D2 mRNAs and activities in WAT from control (C), thyroidectomized (Tx), and T4 + T3–treated thyroidectomized rats. Values are means ± SEM. *P < .05 vs C; # P <.05 vs Tx (n = 4-5/group).

measured by quantitative real-time polymerase chain reaction (qRT-PCR) using Taqman probes (Rn00572183 m1, Rn00581867 m1, Applied Biosystems, Foster City, CA). After normalization to 18S rRNA, the fold-change in mRNA expression was calculated by the $2^{-\Delta\Delta Ct}$ method.

2.4. *Determination of thyroid hormone concentrations*

Thyroid hormone concentrations were determined by radioimmunoassay in plasma, liver, and WAT after extraction and purification [15]. High specific activity T4, rT3, and T3 labeled with [^{125}I] was synthesized as described [15].

2.5. *Statistical analysis*

Mean values (±SEM) are given. Significant differences were achieved by 1-way analysis of variance.

3. Results

3.1. *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). Afterward, we did titration curves using qRT-PCR using Taqman probes (Fig. 1A), confirming that D1 and D2 mRNAs were present in rat epididymal WAT, with D2 mRNA abundance being higher than D1 mRNA (approximately double). White adipose tissue D1 mRNA abundance was less than 1% of liver D1, and WAT D2 mRNA abundance was about 7% of BAT D2 (Fig. 1B, C).

We then analyzed D1 and D2 activities in WAT. First, the kinetic characteristics were determined; for D2: Km = 40 nmol/L T4 and maximal velocity = 3 pmol/h per milligram protein and for D1: Km = 0.35 μmol/L rT3 and maximal velocity = 6 to 18 pmol/h per milligram protein (control and Tx rats, respectively). Type 1 deiodinase activity was higher in perirenal WAT; D2 activity was similar in perirenal and epididymal WAT and lower in subcutaneous WAT (results not shown).

3.2. *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 by measuring T4 and T3 concentrations in plasma, liver, and WAT. Fig. 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, whereas 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, whereas liver T3 concentrations did not fully recover.

Type 1 and type 2 deiodinase mRNA increased in WAT from Tx rats (Fig. 1D, E), and T4 + T3 treatment inhibited them to control values for D1 and to less than 50% of control values for D2 mRNA. Contrary 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.

4. Discussion

Type 1 deiodinase activity was identified in WAT in 1983 together with D2 activity in BAT [8], establishing clear differences between BAT and WAT. Afterward, 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 at both mRNA and activity levels. We used epididymal WAT, the purest of all WAT locations, never converted into BAT under

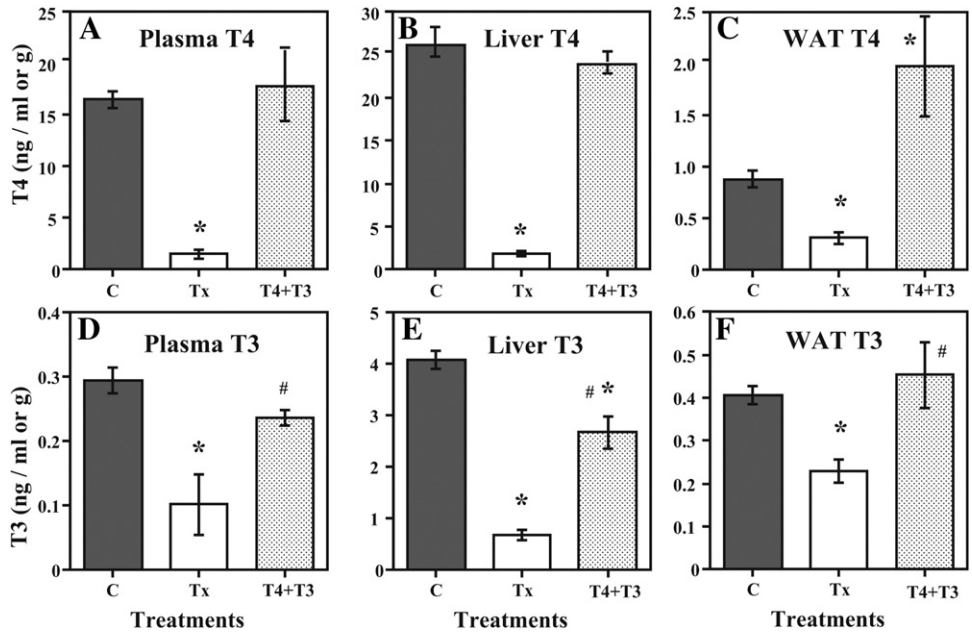

Fig. 2 – The T4 and T3 concentrations in plasma, liver, and epididymal WAT from control (C), thyroidectomized (Tx), and T4 + T3–treated thyroidectomized rats. Values are means ± SEM. *P < .05 vs C; #P < .05 vs Tx (n = 4-5/group).

cold exposure; in contrast, inguinal fat is considered "convertible" adipose tissue, which 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]. Uncoupling protein 1 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 that 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 upregulated in hypothyroidism. White adipose tissue D2 increased as expected in hypothyroidism. White adipose tissue 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 recently showed increased D2 mRNA in parallel to D2 activity levels in human subcutaneous fat [18]. Type 1 deiodinase 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. Type 1 and type 2 deiodinase 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.

5. Conclusions

Type 1 and type 2 deiodinase 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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