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Regional Decrease of Subcutaneous Adipose Tissue in Patients with Type 2 Familial Partial Lipodystrophy Is Associated with Changes in Thyroid Hormone Metabolism

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Background: Familial partial lipodystrophy of the Dunnigan type (FPLD2) presents with a decrease of subcutaneous adipose tissue (SAT) in the limbs and trunk. As thyroid hormones (TH) play an important role in adipogenesis, we studied if SAT from subjects with FPLD2 have changes in the gene expression levels of monocarboxylate transporter 8 (*MCT8*), a TH transporter, and TH nuclear receptors and in iodothyronine deiodinases (DIOs) expression and activities that could affect TH bioavailability and action in white adipose tissue.

Methods: Seven subjects with FPLD2 and 10 healthy controls were studied. Two biopsies of SAT were obtained from each subject, one near the umbilicus and the other from the thigh. Expression of *MCT8*, *DIO2*, *DIO3*, *THRA1*, *THRB1*, and *RXRG* mRNAs were quantified by real-time polymerase chain reaction. DIO1 and *DIO2* activities in adipose tissue homogenates were determined. Serum thyroid-stimulating hormone and TH levels were measured by chemiluminescence.

Results: Subjects with FPLD2 had lower levels of *MCT8* mRNA expression in the thigh than in the abdomen SAT, and lower than in the abdomen and thigh SAT from control subjects. FPLD2 subjects also had higher *DIO2* expression and activity in the thigh than in the abdomen SAT and higher than in controls.

Conclusions: Thigh SAT from subjects with FPLD2 has lower expression of *MCT8* and higher *DIO2* expression and activity than abdominal SAT, suggesting that changes in local TH metabolism may occur in areas with lipoatrophy. *DIO2* expression and activity in SAT suggest that DIO2 can regulate the metabolism and action of TH in human white adipose tissue.

Introduction

Thyroid hormones (THs) regulate adipogenesis and adipose tissue lipogenesis and lipolysis (1). *In vitro* THs induce an acceleration of adipocyte differentiation (2), and several genes involved in the differentiation program of adipocytes are regulated by triiodothyronine (T3) (1,3). THs also upregulate gene expression and activity of lipogenic enzymes in white adipose tissue (4,5), although T3-stimulated lipolysis is higher than T3-stimulated lipogenesis (6). In hyperthyroidism, lipolysis is enhanced in part because the adipose tissue has an increased sensitivity to catecholamines that result from an increased β-adrenoreceptor number and a decrease in phosphodiesterase activity. Both regulations concur to increase cAMP levels and hormone-sensitive lipase activity (7,8).

TH cell uptake is mediated by specific plasma membrane transporters. Monocarboxylate transporter 8 (MCT8) is a TH-specific transporter ubiquitously expressed. Mutations in human MCT8 gene cause a syndrome with severe psychomotor retardation in affected males and a thyroidal phenotype in the affected subjects and the female carriers (9,10), underlining the importance of MCT8 in cellular TH homeostasis. MCT8 is expressed in human subcutaneous adipose tissue (SAT), and although very little is known about its regulation, MCT8 expression in adipose tissue decreases during septic shock (11).

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In humans, approximately 80% of T3 is produced by extrathyroidal enzymatic deiodination of thyroxine (T4). SAT expresses type 1 (*DIO1*), type 2 (*DIO2*), and type 3 (*DIO3*) iodothyronine deiodinases, and DIO1 and DIO3 activities have been reported in human SAT (11). These findings suggest that iodothyronine deiodinases could play an important role in regulating the concentration of T3 in the white adipose tissue.

TH action depends on the tissue distribution and expression levels of thyroid hormone receptors (TRs), which are ligand-regulated transcription factors that bind to thyroid response elements (TREs) of target genes. TRs are encoded by the *THRA* and *THRB* genes, which by alternative splicing give rise to different TR isoforms (12). TRs bind to *TRE*s predominantly as heterodimers with the retinoid X receptor and together they also bind to other regulatory proteins that act as corepressors and coactivators modulating TR transcription function. All the isoforms of TRs are expressed in white adipose tissue (11).

Familial partial lipodystrophy of the Dunnigan type (FPLD2) is characterized by loss of SAT in the limbs and trunk from puberty, accumulation of fat in the neck and face, and predisposition to insulin resistance, leading to complications such as glucose intolerance, dyslipidemia, high blood pressure, liver steatosis, and increased risk of coronary heart disease. FPLD2 results from heterozygous missense mutations in the *LMNA* gene (13), which codes for differently spliced proteins, including lamin A, lamin C, and lamin AΔ10. Lamin A is synthesized as a precursor, prelamin A (14), and subjects with FPLD2 have an accumulation of prelamin A in SAT, which is associated with a reduced expression of several genes involved in adipocyte proliferation and differentiation (15).

Because of the important role of TH on adipocyte differentiation and metabolism, we wondered if the peculiar adipose tissue distribution of subjects with FPLD2 is associated with changes in *MCT8*, *DIO2*, *DIO3*, *THRA1*, *THRB1*, and *RXRG* mRNA expression levels and with changes in DIO1 and DIO2 enzyme activities that could have an effect on TH bioavailability and action in the white adipose tissue of these subjects.

Materials and Methods

Subjects

We have studied seven subjects with FPLD2 due to a missense mutation in LMNA gene (R482W), belonging to a previously published pedigree (16). Ten healthy participants matched by age, sex, and body mass index were used as controls. Anthropometrical, body composition, insulin resistance status, and other relevant clinical data of the participants have been previously reported (15). This study was approved by the ethics review panel of the Conselleria de Sanidade (Xunta de Galicia, Spain) and performed according to the ethical guidelines of the Declaration of Helsinki. Control and FPLD2 subjects gave informed consent for participation in the study and for publication of their clinical, biochemical, and genetic information.

Serum analysis

Serum TH levels were measured by chemiluminescence (ADVIA Centaur, Bayer Diagnostics, Barcelona, Spain) in blood samples taken between 0800 and 0900 hours. Normal reference values for serum thyroid-stimulating hormone (TSH) were 0.41–4.94 mU/L, for free T4 (FT4) 10.94–21.75 pM (0.84–1.68 ng/dL), and for free T3 (FT3) 3.89–6.60 pM (2.52–4.28 pg/mL).

Adipose tissue biopsies

Two biopsies of SAT, 1 cm each, were obtained from each participant under sterile conditions and local anesthesia. One biopsy was taken from subcutaneous deposits 4 cm lateral to the umbilicus and the other from subcutaneous deposits in the lateral side of the thigh. Fat samples were cleaned of visible fibrous connective tissue and blood vessels and were divided into roughly equal portions, one of them was snap-frozen immediately in liquid nitrogen and stored at -80°C until further analysis.

RNA isolation and real-time polymerase chain reaction

Total RNA was extracted from the subcutaneous biopsies using a commercial kit (RNeasy Lipid Tissue Kit; Qiagen, Madrid, Spain) following the manufacturer's instructions. RNA was reverse transcribed using M-MLV reverse transcriptase (Invitrogen, Barcelona, Spain), 1 μg total RNA, 5 μM random hexamers, and 2 mM deoxynucleotides (Ecogen, Barcelona, Spain) in a 20 μL reaction volume.

Expression of *MCT8*, *DIO2*, *DIO3*, *THRA1*, *THRB1*, and *RXRG* mRNAs and the internal control RNA polymerase II was quantified in a thermal cycler (Light Cycler 2.0; Roche Diagnostics, Sant Cugat del Valles, Spain) using specific probes and oligonucleotide primers (Universal ProbeLibrary; Roche Diagnostics). Real-time polymerase chain reaction conditions are available upon request. Results were normalized to the RNA polymerase II gene, using the $2^{-\Delta\Delta Ct}$ method (17).

Determination of DIO1 and DIO2 activities

DIO1 and DIO2 activities were assayed in adipose tissue homogenates prepared (1:8, weight/volume) in 0.32 M sucrose, 10 mM HEPES, and 2 mM dithiothritol (DTT). Because of difficulties during pipetting, the homogenates were centrifuged for 5 minutes at 1000 rpm to separate the lipid cake as upper phase and the infranatant was used for the deiodinase assays. The protein content was 20–50 μg protein/50 μL homogenate used in the assay. Each adipose tissue sample was assayed in duplicates.

For the determination of DIO1 activity, [^{125}I]-rT3 (70,000 cpm/tube), 100 nM rT3, and 2 mM DTT were incubated at 37°C for 1 hour, using 20–40 μg protein/100 μL reaction mixture (18). Results are expressed in pmol per min/mg protein for DIO1 activity.

DIO2 activity was assayed using [^{125}I]-T4 (60,000 cpm/tube), 2 nM T4 + 1 μM T3 (to inhibit D3 activity), 20 mM DTT, and 1 mM PTU for 1 hour at 37°C, using the same protein as above for DIO1 (19). Results are expressed in fmol per h/mg protein for DIO2 activity.

Before each assay, [^{125}I]-rT3 or [^{125}I]-T4 was purified by paper electrophoresis to separate the contaminating iodide. The [^{125}I]$^{-}$ released was separated by ion-exchange chromatography on Dowex-50W-X2 columns equilibrated in 10% acetic acid. The protein content was determined by the method of Lowry *et al.* (20), after precipitation of the homogenates with 10% trichloroethane (TCA) to avoid

interferences from DTT in the colorimetric reaction. The values shown are the mean of 6–10 adipose samples.

Data analysis

Gene expression levels and enzyme activity are shown as median and interquartile range. Serum hormone levels are shown as mean ± standard deviation. Unpaired *t*-test was used to evaluate for differences between the two groups in TSH and THs serum levels. One-way analysis of variance followed by the Tukey–Kramer test for multiple comparisons was used to evaluate differences in gene expression and deiodinases activities. Data analyses were performed with GraphPad Prism 5.00 (GraphPad Software, La Jolla, CA).

Results

No statistically significant differences were observed between control and FPLD2 subjects in serum levels of TSH (1.61 ± 0.91 mU/L control vs. 1.56 ± 0.33 mU/L FPLD2, ns), FT4 (14.83 ± 1.93 pM [1.15 ± 0.15 ng/dL] control vs. 12.15 ± 2.52 pM [1.13 ± 0.19 ng/dL] FPLD2, ns), and FT3 (4.79 ± 0.38 pM [3.11 ± 0.24 pg/mL] control vs. 5.00 ± 0.5 pM [3.25 ± 0.35 pg/mL] FPLD2, ns) and in FT3/FT4 index (0.32 ± 0.05 control vs. 0.35 ± 0.07 FPLD2, ns).

Subjects with FPLD2 had lower *MCT8* and higher *DIO2* relative mRNA expression in the thigh than in the abdominal SAT. Thigh SAT from FPLD2 subjects had lower *MCT8* and higher *DIO2* mRNA expression than thigh and abdominal SAT from controls (Fig. 1A, E). No differences in relative mRNA expression of *THRA1*, *THRB1*, *RXRG*, and *DIO3* were observed between and within groups (Fig. 1B–F).

DIO1 activity was detected in all SAT samples. Although DIO1 activity tended to be higher in thigh adipose tissue from subjects with FPLD2, no statistically significant differences were achieved (Fig. 2A).

DIO2 activity in thigh SAT was higher than in abdominal SAT in FPLD2 and also higher than in thigh and abdomen SAT from controls (Fig. 2B).

Discussion

Subjects with FPLD2 lose adipose tissue in limbs and buttocks and accumulate fat in the face and neck. In our clinical experience, however, when these patients become obese they also accumulate subcutaneous fat in the abdomen. To date,

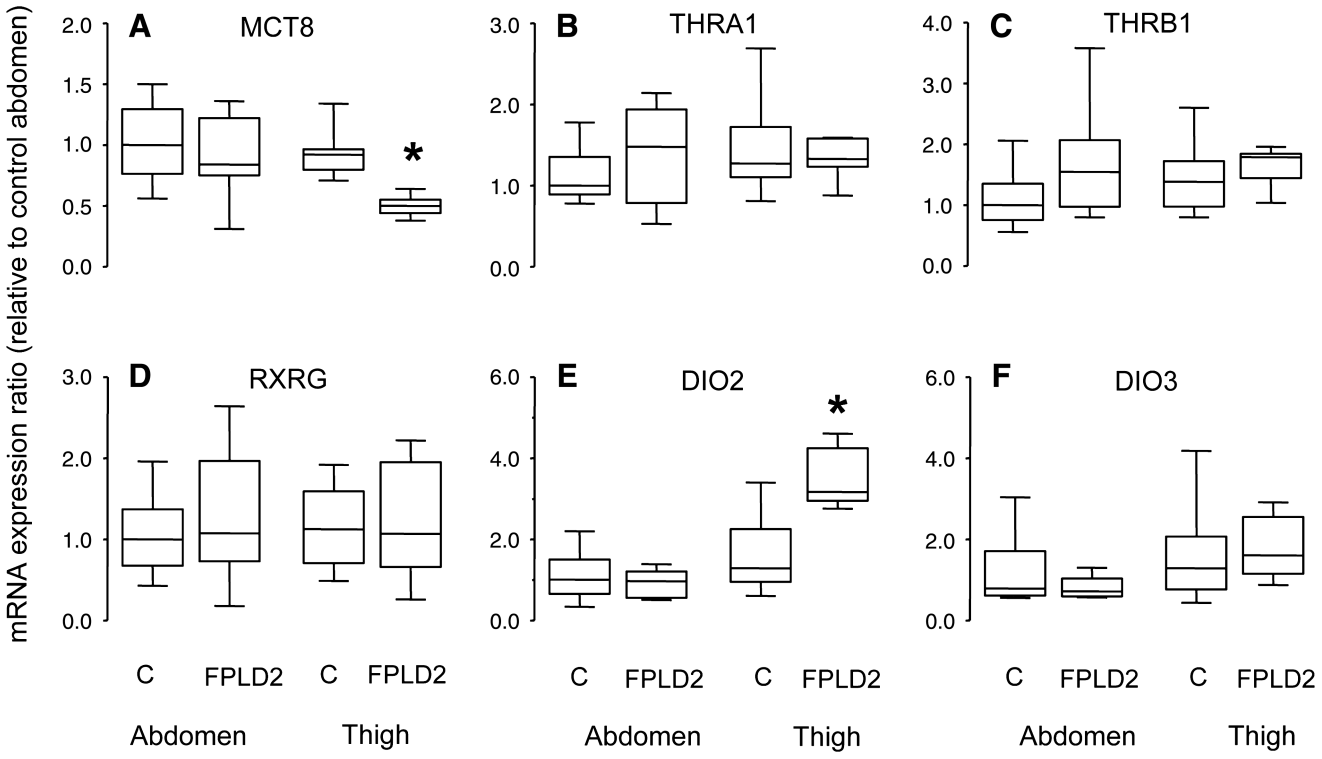

FIG. 1. *MCT8*, *THRA1*, *THRB1*, *RXRG*, *DIO2*, and *DIO3* mRNA expressions were normalized to RNA polymerase II gene. Abdominal gene expression in control subjects was chosen arbitrarily as the reference value and relative mRNA expression in SAT from thigh of controls and from thigh and abdomen of FPLD2 subjects were calculated against it. One-way analysis of variance followed by the Tukey–Kramer test for multiple comparisons were used for statistical analysis. The thigh SAT from subjects with FPLD2 had the lowest relative mRNA *MCT8* expression (**A**) and the highest relative mRNA *DIO2* expression (**E**). No differences in relative *MCT8* and *DIO2* gene expression were observed between abdominal SAT from FPLD2 subjects and thigh and abdominal SAT from controls. No statistically significant differences between or within groups were observed in regional SAT relative expression for *THRA1* (**B**), *THRB1* (**C**), *RXRG* (**D**), and *DIO3* (**F**). Values are shown as median and interquartile range. $^*p < 0.05$ between FPLD2 thigh SAT versus FPLD2 abdomen and controls' thigh and abdomen SAT. FPLD2, familial partial lipodystrophy of the Dunnigan type; C, controls; SAT, subcutaneous adipose tissue; MCT8, monocarboxylate transporter 8.

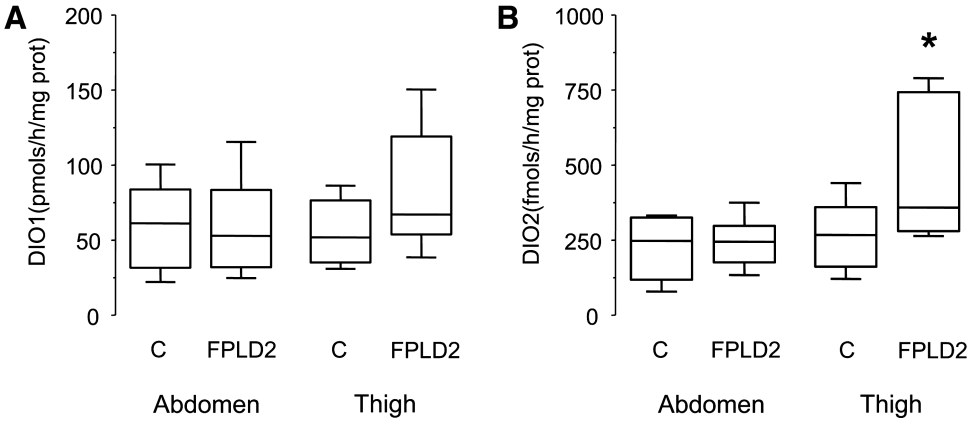

FIG. 2. Type 1 (DIO1) (**A**) and type 2 (DIO2) (**B**) iodothyronine deiodinase activities were determined in abdominal and thigh SAT from control and FPLD2 subjects. One-way analysis of variance followed by the Tukey–Kramer test for multiple comparisons was used for statistical analysis. DIO2 activity was significantly higher in thigh SAT of FPLD2 subjects than in their abdominal SAT and than in controls' abdominal and thigh SAT. No differences were observed in DIO1 activity. Values are shown as median and interquartile range. $*p < 0.05$ between FPLD2 thigh SAT versus FPLD2 abdomen and controls' thigh and abdomen SAT.

the cause of these regional differences has not been identified, but intrinsic differences in the properties of fat cells from specific anatomical deposits could be implicated. Accordingly, we showed recently (15) that, in patients with FPLD2, loss of SAT is associated with a reduced expression of several genes involved in adipocyte proliferation/differentiation and this was found both in genes related to the cellular cycle (protein Rb gene [*RB1*], cyclin D1 gene [*CCND1*], and cyclin D3 gene [*CCND3*]) and in genes involved in adipocyte differentiation (peroxisome proliferator-activated receptor gamma gene [*PPARG2*], lipoprotein lipase gene [*LPL*], and glucose transporter 4 [*GLUT4*]). Also, we have previously reported that abdomen and thigh SAT of control subjects have different levels of expression of *CCND3*, *CCND1*, and *PPARG2* genes, but its significance remains unknown.

According to our previous observations, we have now found regional changes in SAT expression levels of TH transporter *MCT8* and *DIO2* and in DIO2 activity between controls and FPLD2 subjects. These findings suggest that TH bioavailability and actions in adipose tissue could be different in FPLD2 versus control subjects.

Subjects with FPLD2 have lower *MCT8* expression levels in thigh than in abdomen SAT samples, with no differences observed in *THRB1*, *THRA1*, and *RXRG* mRNA expression. Although TH levels in tissue samples were not measured because of the little amount of adipose tissue available for this study, *MCT8* gene is highly expressed in SAT (11) and a decrease in *MCT8* expression might reduce the TH uptake in the adipocytes; these results suggest that the loss of adipose tissue observed in the thigh of subjects with FPLD2 could be associated to lower supply of TH preventing further lipolysis in the already scarce SAT. The loss of adipose tissue in laminopathies and lipodystrophies are related to a reduced differentiation of mesenchymal stem cells along the adipogenic lineage (21); THs act as pleiotropic factors in many tissues during development, by regulating genes involved in differentiation, including adipogenesis (1), and a decrease in TH supply into the thigh mesenchymal stem cells and preadipocytes could be also related to a reduced differentiation and proliferation of the adipogenic lineage.

DIO2 mRNA expression and activity were detected in SAT samples, with the highest levels found in the thigh SAT from FPLD2 subjects. DIO2 regulates the concentration of active TH within cells from different tissues (22) and more than half of T3 inside some tissues can be generated locally under DIO2 activity (23). DIO2 is essential as a local source of T3 in brown adipose tissue (BAT) to facilitate adaptive thermogenesis under cold exposure (24,25). Also bile acids can confer resistance to diet-induced obesity in mice via upregulation of *Dio2* expression in BAT (26). These findings indicate that, at least in rodents, Dio2 has a central role not only in cold-induced thermogenesis but also in adipose tissue metabolism (27). However, adult humans under thermoneutral conditions (28–30) do not have substantial amounts of BAT and the role of DIO2 in white adipose tissue metabolism remains unknown. In rats, *Dio2* mRNA expression is present in white adipose tissue (31), and Ucp1 knockout mice are lean and have increased expression of *Dio2* in white adipose tissue (32). In a previous human study, we reported mRNA expression of *DIO2* but no enzyme activity in white adipose tissue (11). In this study, *DIO2* mRNA expression was detected and we provided evidences that *DIO2* mRNA expression is regulated because SAT from thigh of FPLD2 subjects showed higher levels of expression than the other studied SAT depot, and finally higher *DIO2* gene expression levels were associated with higher levels of enzyme activity. The differences in DIO2 activity between this study and our previous study remain unknown but could be related in part to different methodologies to measure the enzyme activity.

The significance of a higher activity of DIO2 in SAT from the thigh of subjects with FPLD2 versus their abdominal SAT and versus both thigh and abdominal SAT from controls remains unknown. If the decrease in fat TH transporter *MCT8* mRNA expression is related to a lower uptake of TH for the adipocyte, then an increase in *DIO2* expression and activity seems an adaptive response to prevent intracellular hypothyroidism. On the contrary, if the increase in DIO2 activity is a primary

molecular mechanism occurring in the adipocytes from the thigh of FPLD2 subjects, such mechanism would create a situation of local hyperthyroidism and a decrease in TH transporters would limit the uptake of THs and lipolysis. THs increase *LPL* and *GLUT4* genes expression and decrease the expression of sterol regulatory element binding transcription factor 1 (*SREBP-1c*) (1,3). We have previously reported that *LPL* and *GLUT4* mRNA expression were reduced and that *SREBP-1c* mRNA expression did not change in thigh SAT from FPLD2 subjects (15), an argument against the hypothesis of hyperthyroidism in thigh SAT of these subjects. *DIO1* and *DIO3* mRNA expression and activities are stimulated by T3 and no increase in DIO1 activity and DIO3 expression were observed in the thigh SAT of subjects with FPLD2 (22).

Adipocyte precursor cells give rise to white and brown adipocytes and the retinoblastoma protein regulates white versus brown adipocyte differentiation (33) with functional inactivation of retinoblastoma protein causing the transdifferentiation of white into brown adipocytes. The loss of SAT in our patients with FPLD2 is related to a decrease in *RB1* gene expression (15) and to an increase in *PREF1* gene expression, a gene highly expressed in preadipocytes but not in mature adipocytes. The higher DIO2 activity in the thigh adipose tissue from FPLD2 patients could be related to a transdifferentiation of white adipocytes into brown adipocytes that have a high DIO2 activity. UCP1 is a marker of BAT and *UCP1* mRNA expression was not detected in SAT from both controls and subjects with FPLD2 (data not shown), suggesting that the studied SAT samples were white adipocytes.

Conclusion

SAT from subjects with FPLD2 have regional differences in the expression levels of TH transporter *MCT8* and *DIO2* and in DIO2 activity. The thigh from subjects with FPLD2, a body area with significant loss of fat, has lower expression of *MCT8* and higher *DIO2* expression and activity than abdominal fat, suggesting that changes in local TH availability occur in those areas with lipoatrophy. A significant *DIO2* expression and activity were measured in SAT from humans, and although its physiological role remains unknown, it indicates that DIO2 could regulate T3 availability having an important role in human white adipose metabolism.

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Disclosure Statement

The authors declare that no competing financial interests exist.

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